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PARASITOLOGY, THE "STEP-CHILD" IN THE CLINICAL LABORATORY

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It is a wonderful feeling to be a Registered Medical Technologist! When we finish our training, we have an accumulation of heterogeneous facts and theories whirling about in our minds. As we settle down to the practical, every-day life of the routine laboratory, these bits of knowledge fall into proper sequence and order. Theory unites with practical knowledge, and from this union, efficiency in carrying out procedures grows. Some of us "specialize" and become proficient in that specialty. Others are "departmentalized," and thus grow in wisdom in that particular field. From the individual procedures included in the "family" of facts learned in training, the unit often relegated to the position of "step-child" is that of parasitology. Most technologists keep up a certain interest in blood parasitology, but not so with enteric parasitology. Even those who specialize in bacteriology frequently relegate parasitology to the least important procedure in the department. So also do some Schools of Medical Technology. Training time in parasitology is set at a minimum. More emphasis should be placed on the important part the examination of feces plays in the clinical laboratory. Interest should be stimulated by instructors and senior technologists.

To one who is really interested, the disagreeable aspects of enteric parasitology are forgotten when one enters the fascinating microscopic world of fecal material. There are many things to be seen and learned. Examination of pictures and prepared positive specimens are valuable, but they give only a diagrammatic impression. The fallacy of the value of these learned images may be seen many times, when the same classic picture fails to appear in smears of fresh fecal material. Most of the ova of worms are so pictorial that they are easily recognized, even by inexperienced personnel, but many do not have the same appearance in the vital state, compared to that of the preserved state. This is especially applicable to those of some tape-worms, and the cysts and trophozoites of the protozoans. The classic illustrations of the taenia and hymenolepis ova picture the membranous coat of these parasitic eggs. This same gelatinous coating makes visualization of the ova difficult in freshly passed feces. Only a keen, experienced eye will locate them

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among the other fecal elements, unless their number is great. The trophozoites and cysts of Endamoeba histolytica show a great variation in fixed specimens. Most pictures are diagrammatic. Those nicely rounded chromotoidal bars and geometrically placed nuclei portrayed in pictures of Endamoeba histolytica cysts just do not always appear the same in the vital state.

To the eye of a technologist who rarely examines a stool specimen, there are many constituents which are confusing. Plant cells, mucus cells, pollen, and Blastocystis hominis (a yeast) are some of the bodies which may be reported as parasitic elements by an inexperienced examiner. The recognition of non-parasitic forms is as much a part of parasitology as that of true parasites. Many hours must be spent searching the fields of many fecal smears, negative as well as positive, in order that a technologist may become adept in this field. *There is no substitute for experience.*

Another important factor entering into enteric parasitology is the time element. Too little time spent on a specimen is the rule rather than the exception. A truly competent fecal examination requires extreme patience, intense interest, observation of all materials in the microscopic field, and enough time to rule out or determine positive finds. A sample of feces may be rapidly screened, numerous cysts or ova found, possibly one or more types, and discarded with premature reporting. There may be other pathogens present which were not found, because of the predominance of the ones observed. The rhabditiform larvae of Strongyloides stercoralis are frequently commensal with amoebae and/or giardiae. Samples from several areas must sometimes be examined before any of these parasites are observed. A quick examination and the finding of other ova or parasites, with the resulting discard of the feces, may prevent the physician from receiving the information that his patient also harbours strongyloides.

Since many persons have a natural immunity to tissue invasion of some parasites, and are "carriers" only, the clinical symptoms might not result from some parasites reported. The one which is not found in a hasty examination could be his pathogen. If such a patient is treated for one or more species, he may still retain his illness, if another pathogen present is not observed. Anti-parasitic therapy is specific. Treatment for one species may not eradicate another. Therefore, it is the solemn duty of any technologist who examines feces to spend enough time to find, or rule out, multiple parasitic infestation.

In addition to the search for parasites, fecal examinations should include an alert interest in the over-all state of the specimen. A conscientious technologist will immediately notice any appearance of a reverse enteric flora. This is of extreme importance since the advent of "shotgun" antibiotic therapy. Such stool specimens should be cultured, tested for antibiotic sensitivities, and the reverse flora reported. This action might help to prevent a fulminating staphylococcal infection in a debilitated patient, or it could give the doctor a clue to fever of undetermined origin.

Another phenomenon which a careful parasitology technologist will observe and report is the presence of hematoidin crystals. This may

be an aid to the physician in locating the site of bleeding in a patient whose G. I. series do not indicate gastric ulcers.

All fecal specimens submitted for occult blood should be screened for ova and parasites. This takes little time, and the results may be rewarding. In several instances during the past six years, our parasitology unit has found motile trophozoites (in mucus) or cysts of Endamoeba histolytica in stool specimens for which the request was for occult blood. A follow-up of these patients disclosed that all were "ulcer suspects," with symptoms of ulcer, other than bleeding, but who had no positive X-ray findings. Their symptoms disappeared after amoebic therapy. The attending physicians were pleased.

It behooves any medical technologist to go "out of his or her way" to aid a physician. A little extra time spent, or the reporting of a positive result not requested, is greatly appreciated by all medical men.

Some clinical laboratories do not have a regular parasitology technologist. It is not feasible that every laboratory have a technologist who does nothing but examine feces, but even small laboratories should have at least one person who has enough time to spend, and who is willing to become adept in this field. That person should examine fecal specimens immediately. Too many samples of feces are allowed to sit around for hours, or, in some instances, a day or more, before anyone "has time" to examine them. No technologist will allow blood specimens to sit and sit. Why, then, does this happen to parasitology specimens?

This brings about some mention of a mutual understanding between the laboratory and floor nursing service. Nurses are rigidly trained that the doctor's word is "law." If a physician orders three stool specimens to be sent to the laboratory for examination, that order is usually carried out to the "letter of the law." It matters not that the laboratory is closed or on an emergency basis only, and no one is available to examine the specimen. Those stools are sent in when passed, and even though it may be midnight, the charge nurse's duty has been performed! The reports on such specimens are usually of no clinical value, especially if the patient has diarrhea or has been purged. Any technologist who has a deep interest in enteric parasitology has a feeling of regret that these incidents occur.

A smooth correlation between physicians, ward personnel, and laboratory personnel can be established. All doctors desire the best conditions under which work is performed on their patients. They are usually not aware that their orders are carried out at inopportune times. Consultations between the medical staff, the laboratory, and nursing service concerning the optimum timing and manner of collecting fecal specimens creates an appreciative and trusting attitude in all. If the ward attendants and nurses know and understand the reasons *why* they should send stool specimens only at the proper times, they will give full cooperation.

From the smallest laboratory, where one technologist is "jack of all trades," to the largest laboratory which has unit departments, including a bacteriology unit with one or more technologists, the training and experience in parasitology should be as rigid as that in the other fields of medical technology. Most technologists are competent in reading

blood differentials, but there are many laboratories where not a person is really qualified for the accurate "differential" of a microscopic fecal examination. Are these examinations any the less important? The test costs the patient from $3.00 to $5.00, but no real value is received if the specimen is quickly screened by an inexperienced person.

Following are a few suggestions which have been used successfully in the parasitology unit of our bacteriology laboratory. The non-rotating technologist as well as those who rotate through the department must examine fresh and prepared fecal specimens routinely to keep technique fresh in their minds. Therefore, a qualified person is always available to process stool samples immediately after they reach the laboratory.

Care of Specimens:

Ward personnel should be alert to the following facts. Fecal specimens should be sent to the laboratory as soon as possible after evacuation, and handed to a member of the laboratory personnel. Stools should be passed directly into a wide-mouth container, since contamination with urine, tap water, and disinfectant will eradicate trophozoic forms of amoeba, if present. This will render a false-negative specimen if no cysts are present. This procedure holds true in purged specimens as well as those collected at random. They should also understand that fecal specimens should be obtained only during the normal laboratory work day, and that the patient should not have received oil, barium, or bismuth medications.

Laboratory personnel should keep in mind the facts that, if culture is ordered, stools should be immediately plated on the proper media, and then examined for parasites without delay. Feces should first be examined macroscopically. Consistency, presence of blood, mucus, pus, and general characteristics should be noted. At least 8 to 10 different areas of a formed stool, and as many samples of mucus or blood from liquid specimens should be examined before a negative report is sent out. Sufficient time should be spent on each specimen in order to validate reports.

Microscopic examination

Samples for the wet smears should be taken from the *outside* layers of a formed stool. One should not probe into the specimen. Whether formed, mushy, or liquid, any particles of blood, mucus, pus, or tissue present should be selected for the smears. Slides 2 x 3 inches, and cover-slips 22 mm x 40 mm, are most practical for this work. Both the iodine and saline smears can be placed on each end of the slide, without fear of over-running. With an applicator stick, a small bit of fecal material should be stirred first into a drop of 0.85% saline, and then into a drop of iodine on the other end. Place cover-slips on the emulsions, with the length of the cover-slips in a vertical position. Smears should not be too heavy.

With the low power objective of the microscope, scan all the area in the two preparations. Any bright, refractile object should be viewed with the high-dry objective to determine whether it is a parasitic element or an artefact. With experience, the technologist can pick out cysts or amobae easily under the low power. There is a certain "vitality"

radiating from these bodies, which, to the trained eye, distinguishes them from similar but non-living things. Identification of course must always be made under high-power. The ova of Nemathelminthes and Platyhelminthes are large and are more easily identified than the cysts of the protozoans. The shape and type of outer membrane of these eggs are their differentiating characteristics. A brief resume of seven of the most common types of helminth ova found in feces is outlined below.

1. *Necator americanus* (or *Ancylostoma*)—hookworm—ova have a thin, refractile, cell membrane and have no color. These ova have usually developed to the 4 to 16 cell stage of larval cleavage when passed. This gives the interior of the egg a "bumpy" look.
2. *Taenia saginata* (or *solium*) beef and pork tape-worm ova are of identical morphology. The membrane is usually cream-colored to brown. Radial striations occur, forming a curved margin, which frames small hooklets, usually six in number.
3. *Hymenolepis nana*—dwarf tapeworm ova have a double membrane, are colorless, and almost transparent. Between the membranes, small twists or filaments may be seen. This is a specific differentiating characteristic. The edges are not striated. Six hooklets are present.
4. *Enterobias vermicularis*—pin-worm ova are very rarely found in routine feces, except in an occasional specimen passed very early in the morning. A N.I.H. scotch-tape swab, used around the anus during hours of sleep, is more satisfactory. The eggs are oval, flat on one side, colorless, have a double membrane, and generally contain a well-developed larva.
5. *Trichuris trichiuria*—whip-worm ova are much smaller than those of the other round-worms. They have a distinctive shape caused by a "plug" at each end. They are reddish-brown in color.
6. *Ascaris lumbricoides*—ova are brown, and the inner membrane is covered by a scalloped, horny outer coat. The developing embryo may be seen inside. At times, the ova of Ascaris lose the outer shell, or are non-fertilized. This changed morphology should be kept in mind when odd forms are seen which resemble Ascaris ova.
7. *Strongyloides stercoralis*—ova are not found in feces, except in cases of violent purges. These ova hatch in the tissue of the duodenal region, and the rhabditiform larvae are passed out in the feces. The ova are morphologically indistinguishable from those of hook-worm. A point to remember is that, normally, only *larvae of Strongyloides*, and *ova of hook-worm* are found in fresh fecal matter. Hook-worm ova hatch outside the body. Strongyloides larvae are very active, and have a coiling whipping movement.

Concentration of fecal specimens

Several slide concentrations of properly selected material from stool specimens will yield as many, if not more, positive findings as the washing, straining, and concentration of the entire specimen. This is especially true in suspected amoebiasis. These parasites are harboured in the wall of the intestine and therefore are more likely to be found, in formed specimens, in the light layer of intestinal secretion on the cast of feces formed by the rectum. The saturated sodium-chloride flotation

method for the demonstration of helmith ova should be used on all specimens if none of these are found in the slide concentrations.

S/S NaCl Flotation Technique: Belding, 1952

In a small ½ oz. container, emulsify a sample of feces about the size of a walnut in s/s NaCl. Add the NaCl until a meniscus is formed. Place a 2" x 3" slide over the emulsion and let stand for 15-30 minutes. At the end of this period of time remove slide, invert, and apply a coverslip. Examine under low-power objective. This method is particularly good for demonstration of hook-worm ova.

Purged specimens

Any specimen of feces which is positive for Endolimax nana, Endamoeba coli, and/or other non-pathogenic protozoans should be considered "presumptive positive" for Endamoeba histolytica, just as water samples culturally indicating fecal contamination are presumed to contain the typhoid bacillus. Often these pathogens are passed in showers at periodic times, and might not be present in the specimens examined and found positive for the non-pathogenic protozoa. When no forms of Endamoeba histolytica are observed in a specimen which contains one or more other parasites, a light purging of the patient will usually result in the demonstration of this pathogen. In the past six years, we have found Endamoeba histolytica to be present in the purged specimens from many patients whose formed feces contained cysts of other parasites, but in which no Endamoeba histolytica were seen. Only two patients, one with Endolimax nana and one with Iodamoeba bütschlii, passed fluid feces in which no cysts or trophozoites of Endamoeba histolytica were demonstrated.

Purging with Fleets phospho-soda® is an advantage when a satisfactory specimen cannot be obtained, or if daily specimens are not feasible. This procedure can be carried out on out-patient, in-patient ambulatory, or in-patient ward bases. It requires the entire attention of the technologist during the period, but the results are gratifying to the physician. Amoebiasis can be definitely ruled out or determined in a period of about two hours. This is much more satisfactory than the examination of one or more dry formed specimens, obtained at random. Since these cysts and/or trophozoites are excreted periodically, unless diarrhea accompanies infestation, the examination of serial specimens is necessary. One might find a sample from the patient positive on one day, but not on another.

Amoebae, harboured high in the intestinal tract, do not encyst and are usually lysed in passage down to the anus in a formed stool, resulting in a falsely negative specimen. A purge will bring them down in good condition.

In carrying out a purge, all specimens passed, from the first to the last, should be examined. Endamoeba histolytica may invade the sigmoid-rectal area, the cecum, the ascending colon, or the ileum. Giardia lamblia and Strongyloides stercoralis attack the area of the duodenum. Helminth ova may lie anywhere along the intestinal tract. If the first specimen

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of feces passed is not examined, amoebic elements might be missed if infestation is in the rectal area only.

Purging is of great value when "ruling-out" or rapid diagnosis of parasites is requested by the physician. Hospital stay is shortened and out-patient service can be utilized if desired. Most doctors will give permission for this procedure, if the patient's condition does not contraindicate purging, and they appreciate the expedition and validity of the reports. It might be stated that the significance of negative findings in purged specimens is 100% greater than those of a single formed stool, or three or four passed over a period of days.

Methods of identifying protozoans

Endamoeba histolytica—trophozoites—a fresh warm specimen will insure characteristic motility. This animal has a small, rounded, finely granular endoplasm, from which clear, hyaline pseudopods erupt explosively. In the active living state it travels in a rapid, purposeful manner across the microscopic field. Inclusive in the cytoplasm may be one or many red blood cells. The nucleus is delicate, has a centric karyosome, and cannot be seen easily, unless stained.

Cysts are usually round, but may be slightly deltoid in shape. The characteristic number of nuclei is 4, but as many as 6 may be found. Younger cysts may have 1, 2, or 3 nuclei. The chromatoidal bars are also characteristic, but are not always observed. They are not usually present in mature cysts. The nucleus of the cysts of Endamoeba histolytica has a delicate chromatin network and a centric karyosome.

Endamoeba coli—trophozoites are more often larger than those of Endamoeba histolytica, and have a coarse endoplasm from which "dirty" gray, granular, sluggish pseudopods protrude. Some trophozoites of Endamoeba coli, however, very closely resemble the inactive trophozoites of Endamoeba histolytica. This may lead to confusion in diagnosis. The nucleus of the Endamoeba coli trophozoite is coarse and beaded, has an eccentric karyosome, and may be seen in an unstained preparation. These trophozoites ingest bacteria, yeast, and foreign bodies.

Cysts are large, occur in various shapes from round to bizzare. The common number of nuclei is 8, but from 2 to 16 may be present. Each nucleus is surrounded by a coarse framework, the chromatin net is also coarse, and the karyosome is eccentric. Young cysts are usually binucleated, and contain a large glycogen mass, while splinters or sheaths of glycogen are the inclusions found in older cysts.

In differentiating between the forms of these two parasites, one must bear in mind that:

1. Sluggish trophozoites of Endamoeba histolytica may be mistaken for the vegetative forms of Endamoeba coli.
2. The trophozoites of Endamoeba coli have been known to ingest a red blood cell, occasionally.
3. There are a large and a small race of Endamoeba histolytica. Some strains may be as large as Endamoeba coli.
4. Identification should be made only from fresh warm fecal material containing actively motile vegetative forms, or from properly stained cysts.

Giardia lamblia—When present, the cysts of this flagellate are usually found in great numbers. They are oval or round, and contain from 2 to 4 nuclei. They have a distinctive pattern of curved, rodlike inclusions formed by the axostyles and parabasal bodies of the animal. The eccentric karyosomes of the nuclei give these cysts and flagellates a "cross-eyed" appearance. The outer membrane is usually separated from the inner membrane. A glycogen mass is sometimes observed in iodine stained smears. The round forms of Giardia cysts must be distinguished from the cysts of the small race of Endamoeba histolytica.

Staining techniques for use in direct smears of fecal material

For suspending fecal material in direct smears, a contrasting background may be obtained if the physiological saline is made up in 0.5% eosin. This solution gives a nice contrast, but is not as deeply colored as one made in 2%. Lugol's iodine is an excellent preparation, and is much easier to make up than the D'Antoni's iodine. The addition of eosin stain, in a 1% concentration, to Lugol's iodine will result in a di-chromic stain, the iodine content of which will be picked up nicely by all cellular materials, and by the interior structures of cysts and trophozoites. The eosin will not penetrate the cell membrane of living trophozoites. In some instances, the cysts of enteric parasites will not stain well, and detailed characteristics cannot be visualized. Concentrated iodine solutions result in too deep a stain. All technologists experiment periodically, and such testing of various laboratory solutions resulted in our stumbling onto a method by which these stain-impenetrable cysts could be altered so that the iodine entered easily. Basic and acid solutions, routinely used around the chemistry laboratory were tried out. When the 12/N sulfuric acid, used for protein-free-filtrates was tried, the cysts stained in a classic manner. This technique is outlined below.

Procedure: "acidified fecal staining"

A drop of 12/N sulfuric acid is placed on a glass slide. Using an applicator-stick, a sample of feces is emulsified into the acid solution. The feces are allowed to stand in the acid solution for 3 to 5 minutes. A drop of this acidified emulsion is then transferred to a drop of Eosin-Iodine on another slide, and a cover-slip added. Cysts will appear pale greenish brown, and all intra-cellular parts show up in intricate detail. It is important that the acidification is done *first, before* the addition of the iodine. If iodine is added before the acid, the cysts will remain impenetrable to the stain.

Differentiation of the feces in amoebic and bacterial dysentery can be made by observation of cellular material in the stools. The diarrhea of amoebic dysentery produces liquid stools which have a distinctive ptomaine-like odor, while those of bacterial diarrheas have an odor much like strong soda-water. The cellular materials found in bacterial diarrhea are polymorphonuclear cells, in profuse numbers, and in most instances the specimen is not bloody, although some red blood cells may be found microscopically. When present, these blood cells are seen lying free in the material, not clumped. Macrophages are also present in various

quantities. The feces in amoebic dysentery contain few cells, unless a secondary bacterial infection is present. Those which are found are usually degenerated. Bloody stools are often produced. Microscopically, the red blood cells are conglutinated. Charcot-Leyden crystals are usually present.

This paper is presented in an attempt to spur interest in the field of parasitology in relation to the clinical laboratory. It is hoped that it might, in some small way, influence all who take part in the training of medical technologists to intensify the training and experience of these persons in this branch of our profession, and stimulate a positive attitude toward this important diagnostic aid. Some of the procedures and personal observations of the author have been outlined, in order that they might be passed on to others for any assistance they might give. Let's include our "step-child" among the more favored members of the family of medical technology!

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INTERPRETATION OF PROTEIN-BOUND IODINE VALUES*

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The chemical determination of the protein-bound iodine in the blood stream has become a well established, reliable means by which a physician can measure clinically the activity of the thyroid gland. However, in order to obtain meaningful values, the test must be performed on serum from patients who have been adequately prepared. Both the laboratory personnel and the physician should have some common knowledge concerning the interpretation of values and the influential factors involved.

The generally accepted normal range of values for serum protein-bound iodine is 4 to 8 micrograms per 100 ml., although published data on the normal range have been reported as 3.4 to 8.0 micrograms per 100 ml. of serum. Values ranging from 3.0 to 4.0 micrograms per cent are indicative of a mild degree of hypothyroidism which is sometimes associated with chronic infections of the upper and lower respiratory tract and sinusitis. Values ranging from 8.0 to 10.0 micrograms per cent are indicative of subclinical to mild hyperthyroidism.

Low levels exclusive of thyroid malfunction are sometimes encountered in patients with albumin deficit usually associated with nephrosis. Mercurial drugs, including mercurial diuretics and mercurial ointments invalidate the results of the laboratory test for 24 to 48 hours. Also, a drop in protein-bound iodine concentration usually occurs during complications of pregnancy such as threatened abortion.

Elevated protein-bound iodine levels exclusive of thyroid malfunctions are influenced by the following factors: (1) Diets restricted in iodine content may produce increased avidity of the thyroid gland for iodine. (2) Diets rich in sea food may produce elevation for 24 to 48 hours. (3) During normal pregnancy, the protein-bound iodine values may be as high as 10 to 11 micrograms per cent, probably due to alteration in thyroxine-binding to protein. (4) Intravenous pyelograms and treatment with thyroxine and thiouracils interfere with measurement of protein-bound iodine up to 1 month. (5) Gall bladder dyes interfere with measurement of protein-bound iodine for 2 to 6 months.

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LEGAL RESPONSIBILITIES AND LIABILITIES OF PATHOLOGISTS AND MEDICAL TECHNOLOGISTS*

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It is common knowledge that patients are filing suits for negligence and malpractice in ever mounting numbers, and for an increasing variety of reasons. All members of the medical team—physicians, nurses, technologists and others engaged in providing patient care—have responsibility under the law for a complex type of service. Hospitals and clinics, governmental, voluntary and private, are having to assume their share of responsibility for any negligence which may occur. It is not always easy to fix responsibility on any individual member of the team, nor upon the hospital or clinic involved. It behooves each of us to give careful thought to liability insurance and to make certain that we are covered, either directly or indirectly, by liability insurance. We must recognize that the public is beginning to expect that the institution and the medical team will carry adequate insurance and will regard the insurance premiums as being a part of the cost of doing business. This is no trifling matter, for awards as high as $300,000 have been made in the courts of certain states. These high awards are based on the growing realization that insurance is available. The loss of a bread-winner through death, or his inability to resume gainful employment, can result in economic losses of astronomical size. One can see how a jury might react, if the plaintiff, a carpenter, is wheeled into the courtroom and is shown to have major permanent disability. The atmosphere becomes charged if the plaintiff's lawyer draws up a blackboard and calculates the nursing expenses of the next 30 years and the sum which will be needed for his support and for that of his wife and children who sit sadly behind the rail.

Moreover, the techniques of prosecuting claims have been strengthened in recent years. There is a growing tendency to shift the burden of proof more and more to the defendant. This trend arose from the alleged fact that it is sometimes difficult for the plaintiff to secure access to precise information as to what happened. It is also alleged that physicians are reluctant to testify against each other. Lawyers for the plaintiff also point out that a patient under anesthesia is in no position to give a report on accidents which occur in the operating room. These are some of the considerations which have led courts to rule that certain facts speak for themselves—a concept covered by the Latin phrase *"res ipsa loquitur."* The best known example of a fact which speaks for itself is the case where a sponge or a surgical instrument is left in the abdominal cavity following operation. Under normal circumstances this is regarded as *prima facie* evidence of negligence. It is widely agreed that the rule is a just one when applied to a case of that type. However, the application of the rule has been broadened in recent years. For example, some courts have ruled that there is an

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inference of negligence if a patient develops either temporary or permanent paralysis of the lower extremities following spinal or caudal anesthesia. Anesthesiologists object to application of the rule of *res ipsa loquitur* in such a case. They admit that untoward reactions of this type do occasionally occur, presumably because of some sort of idiosyncrasy which could not have been anticipated. Moreover, the patient may not be able to tolerate general anesthesia, in which case a spinal anesthesia is the lesser of two types of risk. It seems unfair to penalize the anesthesiologist for having offered the patient the lessor of two risks. Courts have also applied the rule of *res ipsa loquitur* when certain hazardous types of cardiac or vascular surgery terminate in an unfortunate manner. It is no longer sufficient in such cases that the physician conform to the old rule that he must exercise the skill and diligence which prevails among the physicians of his community. Here, again, the courts seem to be requiring a guarantee of results. The medical view is that this is not a matter of malpractice or negligence. It would appear instead that medical men are being forced to provide certain types of health and accident insurance for the benefit of their patients.

The rule of *res ipsa loquitur* has certain important implications for the pathologist and the medical technologist. The rule has already been applied to cases involving the transfusion of mis-matched blood. Must we look forward to the day when courts will rule that the complications of venipuncture or the uncertainties of analytical methods of the laboratory will be assumed by the courts to be proof of negligence or malpractice? I have no answer to this question.

I now turn from the courtroom tactics and presumption of guilt to the still more difficult matter as to which member of the medical team is legally responsible for a negligent act as defined by statutes or by court decisions. I am not a lawyer, and I cannot hope to comprehend the full significance of these highly technical documents. Fortunately, experienced lawyers have written a number of reviews which have illuminated first one aspect and then another of this complex subject. My bibliography includes a list of interesting articles. My remarks, based largely on these articles, are designed to present the matter as seen through the eyes of a pathologist. I trust that my comments will help to clarify the position of the medical technologist. Naturally, my remarks are not to be regarded as legal advice.

The first thing which impressed me was the apparent fact that negligence, and the liability therefor, is personal. I could cite many cases in which courts have declared that a nurse or a technologist was negligent. This is the personal aspect of the matter. The question will immediately arise in your minds as to who must pay the award, if one is made. In the first place, it seems to be a widely accepted principle of law that an independent operator must pay any awards which are made by virtue of his own negligence. This must be extended, apparently, to include "liability without fault," in certain cases where the rule of *res ipsa loquitur* is broadly interpreted. The independent practitioner includes physicians engaged in private practice; it includes private duty nurses who render bills to the patients who employ them. The physician who serves without salary, as attending physician to a

hospital or clinic, is usually regarded as an independent practitioner. He, or his insurance carrier, is liable for his negligent acts, and it makes little or no difference whether the patient is a paying patient or is indigent.

When we turn to the question of the liability of hospitals, we enter upon a legal field of great complexity. Moreover, the law with respect to hospitals varies from one state to another, and it has been changing rather rapidly in recent years. It seems to be almost a universal rule that proprietary, "for profit," hospitals are liable under the law for the negligent acts of their employees. This follows the rule, widely applicable in the business world, that "the master is responsible for the acts of his servants." This is often expressed by a Latin phrase—*respondeat superior*—"let the superior answer." Apparently, a proprietary hospital is responsible for the negligent acts of the physicians in its employ, as well as for its technical and non-technical employees.

The liability of a governmental hospital for the negligent acts of its employees is variable. In some states it is assumed that a governmental agency cannot be sued without governmental consent. This is a relic of the ancient rule that "a king can do no wrong." However, such immunity of governmental hospitals can be waived by special statutes. Thus it is that suits for negligence can be instituted against federal hospitals and against the governmental hospitals of certain states. Even without such statutes, a few courts have held that governmental hospitals are liable for negligent acts of their employees with respect to paying patients. They did so on the ground that the hospital is performing a "proprietary," not a "governmental" function in so far as it treats paying patients.

When we come to the liability of charitable, "non-profit" hospitals, for the negligent acts of its employees, we enter upon a field of the greatest complexity of all. It is one which varies greatly from state to state, from total immunity and limited immunity to total liability. This complex problem requires the continuing attention of pathologists and medical technologists. Fifty years ago, it was almost a universal rule that charitable hospitals were immune for negligence to indigent patients, commonly on the grounds that the trust funds of a charitable institution could not be diverted from the purpose for which they were contributed. It was usually held in those days that charitable hospitals were also immune from suits by paying patients. It was held, for example, that they too were recipients of charity and that the payments which they make represent contributions to a charitable institution. However, there has been a definite trend away from complete immunity. In only about one-half of the states do charitable hospitals have full immunity from suits arising out of negligence of their employees with respect to patients. In several of these the immunity does not apply to paying patients. In ten or fifteen states the hospitals are subject to full liability. In an equal number, there is partial immunity, depending on circumstances.

The trend away from immunity of charitable hospitals can be illustrated by court decisions in the State of New York. As long ago as 1914, the Court of Appeals of New York pointed out, in its decision,

that a nurse may perform administrative acts as well as professional acts. It was implied that the hospital may be legally liable for negligent acts of an administrative character but not for negligent acts of a professional nature. This distinction is commonly referred to as the Schloendorff rule—a rule named after the plaintiff, Schloendorff, in the aforementioned case. Some years later this rule was confirmed and given specific application in several other New York cases. Recently, however, the courts of New York have broken with precedent, and have apparently abandoned the Schloendorff rule. One clear step in that direction was the case of Berg v. the New York Society for the Relief of the Ruptured and Crippled. This was a case of mis-matching of blood by a medical technologist. The final decision, by the Court of Appeals, was that the negligent act of the technologist was a medical act and that the hospital which employed her was liable for damages. It should be noted that the lower court—the trial court—had already dismissed the suit against the pathologist. The reasons for doing so are not stated in the published decision of the lower court, nor in the text of the summary, prepared for appeal to the higher courts. I have been told, however, that the case against the pathologist was dismissed on the grounds that he too was a salaried employee of the hospital and was not the one who employed and paid the salary of the technologist. The Berg case is of special interest, since it helps to fix the responsibility of the charitable hospitals of New York for the negligent acts of technologists and pathologists employed on salary by such hospitals. The decision, which involves the mis-matching of blood by a technologist, deserves being quoted in its entirety:

> "The blood test was a 'medical act' in the sense that it was preparatory to a transfusion. However, the test was performed not by a physician or nurse but by a technician who was employed and paid by the Hospital and who was so far short of 'professional' status or attainments that only four to six weeks' training was necessary for her job. She was no independent practitioner of a learned profession, working in but not for the hospital. She was a salaried employee doing routine work which required a minimum of skill and training. Therefore, without reviewing or revising the whole Schloendorff v. Society of New York Hospital rule (211 N.Y., 125, 1914) and without determining whether the rule itself has outlived its usefulness, we hold that this particular young woman is liable for her negligence.
>
> "The true holding of Schloendorff was that non-proprietary hospitals were exempt from liability for negligent acts or omissions of physicians and nurses in their professional medical capacities. The bases for Schloendorff were two: First, that such hospitals were 'charities,' and second, that there was no 'respondent superior' situation, since the hospitals were in no real sense the 'employers' of the physicians and nurses who did the work of healing in the hospitals. The first of those Schloendorff bases was later abandoned because it was unrealistic, at least as to paying patients. The second basis, whether or not it may fairly be applied in the case of physicians and nurses who are

salaried members of the hospital's personnel rather than outside professionals called in to minister to patients, is without relevance to cases like this one where the negligent person was a non-professional employee of the hospital.

"Modern hospitals hire on salary not only clerical, administrative and housekeeping employees but also physicians, nurses and laboratory technicians of many kinds. Not only do they furnish room and board to patients but they sell them services which are 'medical' in nature and, though furnished on physicians' orders, are performed wholly by and under the control of the hospitals' salaried staffs. What reason compels us to say that of all employees working in their employers' businesses (including charitable, educational, religious and governmental enterprises) the only ones for whom the employers can escape liability are the employees of hospitals?

"Whatever be the ultimate fate of the Schloendorff rule, this case need not be pushed into the Schloendorff mold.

"The judgment appealed from should be reversed and the judgment of Trial Term reinstated, with costs in this court and in the Appellate Division."

To recapitulate, the "technician" was negligent, the case against the pathologist was dismissed and the hospital was held accountable for the negligent acts of its employees. This case is also of interest in that it declared that a hospital is responsible for a medical act. The Berg case also implied that the distinction between medical and administrative acts—the Schloendorff rule—may be on its way out as far as New York State is concerned.

In 1957, the New York Court of Appeals went all the way in discarding the Schloendorff rule. The case of 1957 is known as the Bing case. A patient was burned as a result of the negligence of a nurse employed by a hospital. The hospital claimed immunity on the grounds that the act was a medical act, not an administrative act. The court ruled, six to one, that the distinction between medical and administrative acts is confusing and unrealistic. The court abandoned the rule and stated that hospitals are legally liable for the acts of their employees while they are acting within the scope of their employment. The court stated among other things, that:

"The conception that the hospital does not undertake to treat the patient, does not undertake to act through its doctors and nurses, but undertakes instead simply to procure them to act on their own responsibility, no longer reflects the fact. Present-day hospitals, as their manner of operation plainly demonstrates, do far more than furnish facilities for treatment. They regularly employ on a salary basis a large staff of physicians, nurses, and interns, as well as administrative and manual workers, and they charge patients more for medical care and treatment, collecting for such services, if necessary, by legal action."

It is thus evident that the Bing case not only discards the Schloendorff rule, it also raises the controversial issue of the corporate practice

of medicine. It is to be noted, however, that the court was confronted with the issue of liability for negligence, not with the question concerning the legality of corporate practice. The court's remarks on the latter were side remarks, known to attorneys as "dicta". Eminent attorneys of New York have expressed the belief that the courts of that state, like those of several other states, would rule that the corporate practice of medicine is illegal, if they were faced squarely with the issue and were thus obliged to give full weight to existing statutes.

It is not yet evident whether the Berg and Bing cases will serve as important precedents when the courts of other states are obliged to rule again on the liability of charitable non-profit hospitals. It is of great importance that the legal responsibility of charitable hospitals be clarified in all states, either through statute or through court decisions. It is evident from what has been said that charitable hospitals still enjoy immunity in many states. If the pathologist operates a laboratory in such a hospital under a lease arrangement and if he pays the salaries of the technologists he would probably be held accountable in most states for negligent acts of the laboratory personnel, on the grounds that such a pathologist is an independent contractor, and that he, as the operator of the laboratory, is responsible for the acts of its employees. There is no unanimity of opinion, however, as to whether the pathologist who operates on a percentage of total or net income of the laboratory is also to be regarded as an independent contractor. In any case, the most urgent question, from the standpoint of the technologist, is the question of who is liable if the hospital enjoys immunity and if the pathologists and technologists have the status of hospital employees. Does the injured patient have any recourse at all under the law? I have consulted two eminent attorneys who have had long and extensive experience with problems of malpractice and liability. One said that a court might rule that the patient has no redress whatever under the conditions cited. He went on to say that nobody would institute a suit against a technologist, because, he said, the technologists "have no money." He might have said the same thing about the salaried pathologists of many hospitals. In any case, the answer is unsatisfactory. It is not likely that the public will continue for long to permit this evasion of responsibility. The trend of the times are in the direction of providing security through some technique of spreading the risk. There seems to be a trend for the courts to whittle away the immunity of charitable hospitals and to require them, or their insurance carriers, to assume financial responsibility for the negligent acts of their employees. In the meantime, however, both pathologists and technologists will do well to make careful enquiries regarding their coverage for liability insurance. It should be recognized that the insurance policy of the hospital may merely provide protection to the hospital in case the courts decide that the hospital itself is liable. Such a policy may provide no protection whatever to the technologists, if they were named as defendants in the suit and if the hospital were declared to be immune against suit.

In conclusion, I believe that the entire matter of liability and liability insurance should be given close and continuous study by pathologists

and medical technologists. It is a study in which we might well decide to collaborate.

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METHOD FOR MICRO-CALCIUM USING THE COLEMAN FLAME PHOTOMETER†

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The use of flame photometry in the determination of sodium and potassium in body fluids has proven so feasible, rapid, and accurate that the desirability of extending these advantages to the estimation of calcium in serum has attracted widespread attention in the past few years.

Whereas all investigators have agreed that the flame photometric determinations of calcium surpass conventional methods in speed and convenience, as well as in preventing loss of the ion by repeated washing of the precipitated salt, many of them have reported the interference of sodium, potassium, and phosphate.[1-4] Other investigators have felt that the problem of sodium and potassium within physiological ranges is negligible in routine calcium determinations on the clinical level, provided that the standards* used contain these ions in the concentration usually encountered in serum.[2-5]

Humoller and Walsh[6] made calcium determinations on the Beckman DU with an acetylene-oxygen flame attachment using direct flame photometry. They found, "Sodium and potassium definitely affect the luminosity of calcium in flame photometry. But the effect is only significant when the luminosity of calcium in the presence of these ions is compared with that obtained in their complete absence. It will be noted in Fig. 1 that changes in sodium and potassium concentration within the physiological range have only a slight effect on the luminosity of calcium and for routine studies can be ignored if the standards are prepared as specified."

Mixed Stock Standard

This solution contains 150 meq/1 sodium, 5 meq/1 potassium and 5 meq/1 calcium per liter. It is prepared by dissolving 0.2503 g. of iceland spar in 25 ml. of 2N HCl in a liter volumetric flask and diluting to approximately 500 ml. with distilled water. After 8.7690 g. of sodium chloride and 0.3728 g. of KCl are dissolved in this solution, it is diluted to volume with distilled water. When the solution was stored in tightly stoppered polyethylene bottles, no changes in composition was detected even after months of storage.

Recovery studies employed the addition of 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, meq Ca/lt serum. They received a linear response when plotting concentration vs luminosity.

Phosphates have been found to depress the calcium luminosity in flame photometric procedures. Observations pertinent to this problem were contributed by Chen and Toribara.[3] They state that the presence of protein prevents, in part, the inhibitory action of phosphate. Therefore an extremely large amount of phosphate must be added to serum to cause any depression on calcium emission. In flame photometry, a five-fold increase of phosphate resulted in an insignificant decrease in the value of calcium. They believe that the usual amount of phosphate

* This is one of the reasons we prefer to use Lab-trol (Dade Reagents) for our standard. It contain the sodium, potassium concentration usually found in serum.

† Read by title, 27th Annual Convention of ASMT, Phoenix, Arizona, June 1959. Received for publication February 1959.

in serum may be ignored but the problem of sodium still presents itself.

Calcium emission lines are much less intense than those of strong emitters such as sodium. Marquardt, Cummins, Phillips, and Fisher[7] ran experiments involving the sodium problem and found that the presence of abnormally high or low amounts of inorganic phosphorus, sodium, potassium, magnesium, and total protein did not significantly affect the values for calcium as measured by flame photometry. Thanks to the work of these men we can eliminate the above problems.

Validity of Coleman Flame Photometer

In order to assess the validity of the Coleman simple method, using the flame photometer, in our laboratory the procedure was applied to various chemistry control sera obtained commercially. The results are given in Table I.

The Coleman flame photometric method was also compared with a standard chemical method, Ferro and Ham method, American Journal of Clinical Pathology, July, 1957 and the comparisons obtained are given in Table II.

TABLE I

Comparisons on Known Values

Specimens	Known Values Stated By Manufacturers	Macro-Coleman Value	Micro-Coleman Value
Lab-trol	5.13 meq/l	5.1 meq/l	5.1 meq/l
Versatrol	5.1 meq/l	5.1 meq/l	5.0 meq/l
Lab-trol	4.98 meq/l	5.0 meq/l	4.9 meq/l
Patho-trol	4.08 meq/l	4.0 meq/l	3.95 meq/l

TABLE II

Comparisons of Coleman Flame Photometer to Ferro & Ham Precipitation Method

Specimens	Coleman Flame Photometric Method	Ferro and Ham Precipitation Method
#1	4.5 meq/l	4.6 meq/l
#2	4.0 meq/l	4.0 meq/l
#3	4.8 meq/l	4.7 meq/l
#4	4.7 meq/l	4.7 meq/l
#5	5.1 meq/l	5.0 meq/l
#6	5.25 meq/l	5.2 meq/l
#7	4.4 meq/l	4.3 meq/l

I Method

Two basic methods for calcium determinations are given in the Coleman manual.[8] The simple method is adequate for diagnostic tests on sera run in clinical laboratories. However, for maximum accuracy in research determinations and in diagnostic evaluation of sera with an abnormal sodium concentration, the advanced method which corrects for strong sodium semissions, should be used. Coleman[8] states that their calcium procedure gives an accuracy within 0.10 meq/1 calcium. "This is believed adequate for diagnostic purposes in the clinical laboratory."

The following is a rapid, quantitative, flame photometric procedure for micro-calcium with no preliminary treatment of the serum sample. It has been adapted from the macro-method described in Coleman's manual.[8] All the tests were performed in duplicate and the average difference

in seventy tests was 0.06 meq/l.

II Collection of Serum Sample

Using the standard technique for finger puncture fill three to four micro-blood collecting tubes.* Place the tapered end to the free flowing blood and fill to approximately 4/5. After collection, place crit-o-caps on the capillary tubes and centrifuge for five minutes at 2000 rmp. Separate the cell pack from the serum, this will yield approximately 0.3 cc of serum.

III Reagents

Reagent 5 (0.02% Sterox)**.....Flush reagent
Reagent 12 (6.0 meq/l sodium and 0.02% sterox).....Zero point
Reagent 15 (1.5 meq/l sodium, and 0.05 meq/l potassium and 0.02% sterox).....Diluent
Lab-trol Standard (dilute 1:25 with reagent 15).....Standard***

IV Equipment

Coleman Flame Photometer Model 21
Calcium filter (cat. 21-205)
Direct reading scale panel (Cat. 21-400)
Four 2.5 ml Coleman #21-310 micro-sample container
Acupette 0.1 ml TC 20° ± ½%
Stoppered graduated flasks 2.5 cc†

V Procedure

a) With an acupette measure exactly 0.1 ml of serum into a 2.5 ml volumetric flask or 5 ml graduated cylinder with stopper.
b) Fill to 2.5 ml mark with Reagent #15.
c) Mix well, pour into a properly labeled 2.5 ml micro-sample container.
d) Into a properly labeled 2.5 ml micro-container pour Reagent 12 and Lab-trol Standard. Also pour into a micro container or 10 ml beaker, Reagent 5 the flush solution. Use the flush solution between all changes of micro-containers on the atomizer.
e) Proceed as directed in the Coleman manuel[8] pg. 34. Place the Ca filter in the filter slot with the symbol "Ca" toward the operator. Set control marked GALV Coarse ⅓ turn from counter-clockwise end and approximately center GALV Fine. Set Steadiness Control knob in front of filter slot at 10.
f) Place the beaker containing Reagent 12 in the sample carrier of the Flame Photometer and set the galvanometer index to zero.
g) Replace beaker with the beaker containing Lab-trol Standard. While atomizing this reagent set galvanometer index to the value of calcium listed on the Lab-trol box with the galvanometer knobs on the measuring instrument.
h) Repeat step f (with reagent 12) and readjust the galvanometer to exactly zero with controls marked BLK.
i) Repeat step g (with Lab-trol Std.) and reset the galvanometer index

* 75 mm long 3.9-4.1 mm O.D., 1.8-2.2 mm I.D., Plain.
** Sterox is a non-ionic detergent used as dilvent and atomizer flush in flame photometry.
*** Calcium standard upon standing tends to precipitate. Lab-trol proved to be more satisfactory, stable, and repetitive.
† A 5cc graduate cylinder is available or a 2.5 ml graduated centrifuge tube with a penny-head stopper produced by Corning Glass Co.

to exactly the value of Lab-trol with the controls marked GALV.

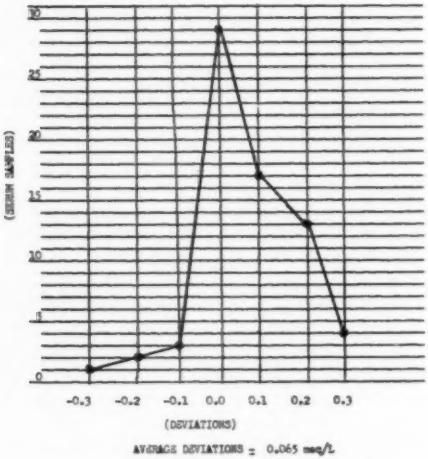
j) Replace the beaker of Lab-trol with the one containing the diluted serum sample. Atomize this solution and record the calcium concentration in meq/l from the Direct Reading Scale.

k) Flush atomizer by atomizing reagent 5 for 10-15 sec.

Studies

Venous blood was taken along with the four micro-blood collecting tubes from finger punctures on the same patient. A macro-Calcium was run on the venous blood and a micro-Calcium was run on the serum obtained from the finger punctures. A total of seventy comparisons were made and are presented in Fig. I in terms of the number of serum samples and their deviations from one another.

Fig. I
MACRO-MICRO CALCIUM COMPARISONS
(SERUM SAMPLES vs. DEVIATIONS)



In order to exemplify reproducibility and per cent recovery of a known added calcium in the analyses of human sera, each of twenty serum samples were analyzed in duplicate with and without the addition of varying quanities of calcium. The recoveries were excellent by an average recovery of 100.4% in the macro-Calcium and 98.7% in the micro-Calcium. The results are listed in Table III.

Common Source of Error

Purity of Water—the water must be free of cations. Dilution of the sample with distilled water having a cation content different from that of the water used in preparing the reagents is a major source of error in any flame photometry.

Technique

Accuracy of the method is primarily dependent on precision in pipetting and diluting to the correct volume, this is assuming that any errors

TABLE III

Recoveries of Additions of Calcium to Various Serums (Represented in meq/L)

Macro-Coleman (venipuncture)

Serum	Ca Present	Ca Added	Ca Found	Percent Recovery
1	4.6	1.016	5.7	101.4
2	4.3	2.032	6.4	101.1
3	5.0	2.54	7.6	100.7
4	4.8	4.064	8.8	99.3
5	4.7	0.508	5.2	99.9
6	4.4	1.524	6.0	101.5
7	4.75	3.556	8.3	100.0
8	4.6	5.08	9.7	100.2
9	4.4	2.032	6.45	100.3
10	4.7	3.048	7.8	100.6

average recovery = 100.4%

Micro-Coleman (finger puncture)

1	4.5	5.08	9.4	98.1
2	4.4	1.524	5.85	98.8
3	5.0	2.54	7.6	100.7
4	4.8	4.064	8.75	98.7
5	4.8	2.032	6.7	98.0
6	4.4	2.54	6.75	97.1
7	4.6	1.016	5.7	100.1
8	4.6	2.032	6.5	98.5
9	4.4	2.54	6.8	98.5
10	4.8	4.064	8.8	99.3

average recovery = 98.7%

in the normal operation of the Coleman Flame Photometer and Coleman Jr. Spectrophotometer are corrected for and the instruments are in perfect operating condition.

Discussion

Table I and II show the validity of the Coleman Flame Photometric method in comparison with a standard chemical method and commercial chemistry control sera. The calcium results obtained suggest that flame photometric determinations are accurate and reproducible. The time required for a simple calcium determination is a matter of minutes.

Summary

A rapid and reproducible micro-method for the estimation of serum calcium by direct flame photometry has been described and evaluated.

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A HANDY "PICK-UP TRAY"

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A handy "pick-up" tray can be made by utilizing a plastic silverware tray. These can be purchased in most stores which sell kitchen utensils and are relatively inexpensive, light weight, sturdy and easy to keep clean.

Such trays are made in both rigid and flexible plastics, the rigid type being somewhat better for this purpose, although the flexible ones could certainly be used. Most of the trays, which measure approximately 10″ x 13″ and are 2″ deep, have five sections: three short and one long lengthwise of the tray, and one short section crosswise.

By drilling holes in wooden blocks to accommodate the various vials used for collecting blood specimens, an antiseptic solution bottle, et cetera, and fitting these blocks, which can be painted if desired, into the sections of the tray, a very useful venipuncture collection tray can be made. There is ample room for cotton, syringes, needles in needle tubes, and so forth, in the sections of the tray not containing the wooden blocks.

If a "pick-up" tray for capillary blood samples is required, it is only a matter of fitting the sections of the tray with a wooden rack to hold the blood pipettes in a rigid horizontal position, a notched rack to hold slides, and a wooden block to hold the bottles of various diluting fluids.

The plastic trays come in white and various colors, which could be utilized in some form of color coding as regards the use of the tray.

A CAPILLARY SAMPLING TECHNIQUE FOR THE DETERMINATION OF CHOLINESTERASE ACTIVITY IN RED CELLS AND PLASMA*

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With the increasing use of organic phosphorous insecticides and the possible use of nerve gases, routine determinations of the effects of such compounds on cholinesterase activity in red cells and in plasma have become a practical necessity. The measurement of serum cholinesterase activity has also been used as a test for liver function.[4, 5]

Michel[3] in 1949 published an electormetric method for the determination of red blood and plasma cholinesterase using human blood. The method was developed to utilize standard laboratory equipment rather than the more complicated manometric or titrimetric techniques. The method required five or more milliliters of blood obtained by venipuncture, necessitating sterile needles and equipment as well as trained personnel. Several determinations on the same individual require successive venipunctures as well as the need for a laboratory nearby for the proper handling and analyses of venous blood samples. Although the Michel macro-method is excellent for occasional analyses, a micro method was needed for large scale screening, following diurnal variations and as a routine diagnostic procedure. Marchant[2] in 1952 published a micro-method using capillary sampling tubes, but the washing of red cells required special micro equipment and was time consuming. The technique presented here avoids these difficulties by using unwashed red cells. Standardization of the centrifugation of the capillary tube containing the blood sample is employed to control the fraction of plasma untrapped and the red cell mass. The micro-method for determining the cholinesterase activity is essentially an electrometric method using capillary blood samples and unwashed red blood cells.

The principle of the method depends upon the hydrolysis of acetylcholine by the enzyme cholinesterase and the liberation of acetic acid which is measured in terms of pH change by enzymatic activity over a given period of time. The change in pH is measured with the glass electrode in a Beckman pH meter. (The change in pH is then corrected for non-enzymatic hydrolysis according to reference table prepared by Michel) and the cholinesterase activity is expressed in pH units per hour. Table 2.

METHOD

A. Materials and Equipment:

1. pH meter, Beckman, Model G
2. Water bath which can be regulated at 25° C ± 0.5° with stirrer for mixing
3. Stop watch
4. Centrifuge—International #1 or #2 with tachometer
5. Micro burner for sealing capillary tubes

* Read by title, 27th Annual Convention of ASMT, Phoenix, Arizona, June 1959. Received for publication February 1959.

6. Small test tubes for holding capillary tubes in centrifuge
7. Capillary tubes—size 1.5-2.0 × 100-150 mm. 2 vials of more, 100 or more tubes per vial
8. Lancets—individual, sterile, disposable
9. Ampule file
10. Forceps, 4-5 inches long—optional
11. 5.0 ml. beakers—2 for each determination
12. Small stirring rods about 1¼" long
13. Sahli hemoglobin pipettes, calibrated
14. Suction tubing with mouthpiece for the above pipettes

TABLE 2

Formula for Calculating ChE Activity in Units of pH/hr.

$$\Delta \text{ pH/hour} = \frac{(pH_1 - pH_2 - b)\, f}{t_2 - t_1}$$

Where pH_1 —initial pH
pH_2 —final pH
t_1 —time of adding Acetylcholine
t_2 —time of reading pH_2
b —non-enzymatic hydrolysis correction
f —correction for variations in pH/hr.

Correction Factors for Use in Formula Above

Plasma Corrections					Red Cell Corrections				
pH_2	—	b	x	f	pH_2	—	b	x	f
7.9		0.09		0.98	7.9		0.03		0.94
7.8		0.07		1.00	7.8		0.02		0.95
7.7		0.06		1.01	7.7		0.01		0.96
7.6		0.05		1.02	7.6		0.00		0.97
7.5		0.04		1.02	7.5		0.00		0.98
7.4		0.03		1.01	7.4		0.00		0.99
7.3		0.02		1.01	7.3		0.00		1.00
7.2		0.02		1.00	7.2		0.00		1.00
7.1		0.02		1.00	7.1		0.00		1.00
7.0		0.01		1.00	7.0		0.00		1.00
6.8		0.01		1.00	6.8		0.00		0.99
6.6		0.01		1.01	6.6		0.00		0.97
6.4		0.01		1.02	6.4		0.00		0.97
6.2		0.01		1.04	6.2		0.00		0.97
6.0		0.01		1.09	6.0		0.00		0.99

B. Reagents:

1. Heparin, 20 mg. per ml.
2. Sodium chloride
3. Potassium chloride
4. Potassium dihydrogen phosphate
5. Sodium Barbital, anhydrous, M.W. 206.18
6. Acetylcholine chloride, Merck 100 mg. ampules
7. 0.1 N HCl
8. 0.1 N NaOH

C. Solutions:

1. Red cell Buffer: 0.02 M sodium barbital (4.1236 g.); 0.60 M KCl (44.730 g.); 0.004 M KH_2PO_4 (0.5446 g.). For one (1) liter of buf-

fer the reagents are dissolved in 900 ml. of distilled water and the pH adjusted to 8.10 with 0.1 N HCl. It is then made to volume, mixed well, and *stored in the refrigerator until needed.* It is advisable to make up only 500 ml. of buffer solution at a time and make it up every two weeks.

2. Plasma Buffer: 0.006 m sodium barbital (1.2371 g.); 0.001 M KH_2PO_4 (0.1361 g.); 0.30 M NaCl (17.535 g.). For one (1) liter of buffer, the reagents are dissolved in about 900 ml. of distilled water, and the pH is adjusted to 8.00 with 0.1 N HCl and the solution is diluted to volume. This solution should also be made every two weeks.

   The stock buffer solutions must be refrigerated when not in use, and aliquots may be taken as needed for the daily work.
3. *Acetylcholine chloride substrate for red cells: 0.11 M acetylchloline chloride (2,000 g. in 100 ml. of distilled water).
4. Acetylcholine substrate for plasma: 0.165 M acetylcholine chloride (3.000 g. in 100 ml. of distilled water).

PROCEDURE

Two glass capillary tubes (for each subject) are dipped in a solution of commercial heparin (10 or 20 mg. per ml.) allowing the heparin to rise about 8 mm. in the tubes. They are then removed and inverted to permit the heparin solution to flow by gravity through the tube and out of the opposite end. A freely flowing drop of blood is obtained from a lanced puncture of the finger tip or ear lobe and allowed to flow into each capillary tube by gravity and capillarity to within approximately one centimeter from the end. *Care is taken to avoid inclusion of air bubbles in the blood columns.* The end of each capillary tube that is free of blood is sealed carefully in the flame of a micro-burner or alcohol lamp, care being taken not to coagulate the blood. The tubes are placed in a horizontal position and allowed to cool for 5 minutes, and then are placed in separate, marked test tubes (100 × 10 mm.) and centrifuged for 20 minutes at 2000 rpm in a No. 2 International centrifuge. Thus, the cells are packed in layers with the white cells separating the red cells from the plasma. The sealed end of one tube is scored with an ampule file about 1-2 mm. from the end of the tube and the tip is broken off. The tube is scored again below the white cell layer and broken cleanly, separating the red cell portion from the white cells and plasma. The duplicate capillary sample is kept for emergency use until results are obtained.

Determination of Activity in Red Cells:

Each capillary tube of blood is sufficient for duplicate determinations on both red cells (unless severe anemia be present) and on plasma. With an accurately calibrated Sahli hemoglobin pipette, the red cells are drawn up to the 0.02 ml. mark directly from the capillary tube, and excess blood is wiped from outside of the pipette. From the Sahli pipette the cells are expelled gently into a 5 ml. beaker containing 1.0 ml. distilled water, keeping the tip of the pipette well below the surface of the liquid. The pipette is rinsed three times with the supernatant water in the beaker before hemolysis is advanced. The cells and water are mixed well with a small stirring rod and allowed to stand for 10-15

* Unstable, must be made fresh daily in small quantities.

minutes to permit complete hemolysis. The stirring rod is left in the beaker for future mixings. To the hemolysate is added 1.0 ml. of red cell buffer, mixed well and placed on a tray in a water bath for 10 minutes at 25° C. After equilibration the initial pH (pH_1) is taken on the Beckman pH meter to the nearest 0.01 pH unit and recorded. Then 0.2 ml. of 0.11 M acetylcholine chloride sol. is added with mixing and the time recorded with a stop watch. The enzymatic action is allowed to proceed for one hour after which time the pH (pH_2) is taken, and recorded. The pH_2 reading is subtracted from the pH_1 reading and the results recorded as ΔpH units per hour. See table 2 for correction for non-enzymatic hydrolysis and factor to correct for change in activity with pH_1.

Determination of Activity in Plasma:

For the duplicate determinations, the plasma portion of the capillary tube is scored and broken above the white cell layer and the leucocytes are discarded. The plasma is taken up in a Sahli pipette to the 0.02 ml. mark and transferred to a 5.0 ml. beaker containing 1.0 ml. of distilled water. After mixing with a small stirring rod, 1.0 ml. of plasma buffer is added, and the procedure is carried out as for the red cell determination using 0.165 M acetylcholine chloride solution as a substrate.

RESULTS

Cholinesterase activity was determined on red blood cells on 31 normal human subjects by both the Michel macro method[3] and the capillary micro method described above. The average red blood cell cholinesterase activity and its standard error for the capillary blood by the micro method was 0.81 ± 0.03 Δ pH/hr., and for the venous blood by the Michel method it was 0.75 ± 0.02 Δ pH/hr. The difference in average red blood cell cholinesterase activities, 0.06 ± 0.01 Δ pH/hr. is significant. The results are shown in Table 1.

In spite of the significant difference in the averages of ChE activity by the two methods, there is a high degree of correlation between the methods, ($r = 0.88$ on 31 pairs of observations) based on a range of cholinesterase activities from 0.30 to 1.00 Δ pH/hr. Chart I.

The variability[1] of the two methods indicated that each observation by the capillary method showed a standard deviation of ± 0.05 Δ pH/hr., while by the Michel method it was ± 0.04 Δ pH/hr., There is no significant difference in these two measures of variability.

Plasma cholinesterase activity was determined on 53 human subjects using the micro sampling method. The average for the group was 0.96 ± 0.11 Δ pH units per hour, ranging from 0.64 Δ pH units to 1.15 Δ pH units.

A statistical analysis based upon observations of red cell cholinesterase activity obtained at monthly intervals for 8 months from a group of 19 men handling nerve gases indicates that the expected variation of a red blood cell cholinesterase activity value as determined by this micro technique is about ± 0.07 Δ pH units per hour.

The evaluation of statistically significant changes in blood cholinesterase activity after exposure to insecticides or nerve gases can be obtained by testing the unknown value against its pre-exposure control

TABLE 1

Cholinesterase Activity in Human Red Blood Cells

Comparison of Results Obtained on Capillary Blood with Michel's Macro Method on Venous Blood

No.	RED CELL △pH/HR.	
	Venous Blood Michel Method △pH/hr.	Capillary Blood Capillary Method △pH/hr.
1	.73	.80
2	.74	.77
3	.83	.95
4	.83	.97
5	.78	.86
6	.88	.92
7	.52	.59
8	.82	.83
9	.80	.81
10	.67	.67
11	.77	.89
12	.88	1.04
13	.69	.83
14	.73	.77
15	.75	.87
16	.80	.88
17	.51	.73
18	.92	.96
19	.30	.32
20	.80	.80
21	.74	.79
22	.64	.68
23	.64	.66
24	.69	.89
25	.82	.95
26	.64	.66
27	.72	.73
28	.82	.86
29	.81	.85
30	.92	.90
31	1.00	.89
Mean △pH/hr.	0.75	0.81
S.E.	0.024	0.025
S.E. of difference	0.012	...

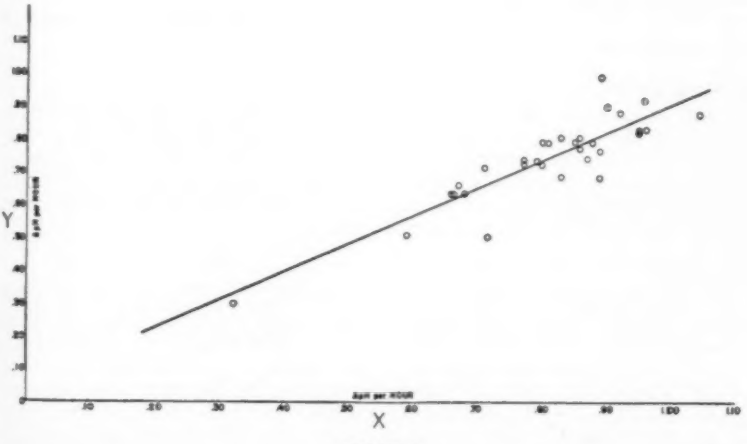

CHART 1

Regression of Michel method (Y) on capillary method (X) of determining Cholinesterase activity.
Prediction formula $Y = 0.054 + 0.86X$ S.E. of estimate = 0.067

level. A suspected low cholinesterase activity, in order to be significant must be less than this average by 0.11 Δ pH/hr. if there are three control values, by 0.14 Δ pH/hr. if there are 2 control values, and by 0.16 Δ pH/hr. if only one control value is obtained. A single observation lower than its limiting value as observed by the above statistical analysis indicates a significant change in the cholinesterase activity level for that individual. Any initial control value below 0.58 Δ pH/hr. indicates a cholinesterase level significantly lower than the normal average.

While the method has a specific application for the detection of insecticide poisoning it may be used clinically in the differential diagnosis of liver diseases with jaundice. In obstructive jaundice the serum or plasma cholinesterase value is normal while in hepatitis, cirrhosis, or liver damage after general anesthesia, the values are decreased. After local or spinal anesthesia the serum or plasma cholinesterase values are also decreased which is thought to be a stress phenomenon. As a surgical guide, the cholinesterase value may be a prognostic measure where surgery would cause stress on the already depleted cholinesterase reserves.

DISCUSSION

The use of capillary tubes for micro-sampling and the determination of cholinesterase activity on packed red cells from which the plasma has not been washed appears to be a suitable technique when compared to the standard Michel method, although the absolute mean values for the two techniques may not be identical. Statistically, the difference between the mean is significant; however, it is of greater importance that they are well correlated and that the standard error for both techniques is alike. The confidence gained in the use of the simpler technique has permitted continuous use of the method on over 1200 samples from approximately 150 subjects with an accuracy consistent with the method as described here.

Generally subjects have preferred to have their finger punctured rather than to have blood drawn from the antecubital vein for repeated tests on the same individual.

Anticoagulants:

It has been found that heparin has no influence on the cholinesterase activity of the blood whereas cholinesterase is inhibited by fluoride, oxalate, citrate, arsenite and pyrophosphate. Presumably these effects are due to a removal of Ca^{++} or Mg^{++} which are said to be activators of cholinesterase. Serum and plasma have comparatively the same activity and values can be used interchangeably.

Substrates:

The pharmacological properties of acetyl-B methylcholine (called mecholyl in the form of its chloride) are qualitatively similar to those of acetylcholine. The former compound is less readily hydrolyzed by the pseudo (non-specific) cholinesterase found in plasma and in serum and has been used as the substrate for whole blood cholinesterase studies; on the other hand, benzoyl choline is hydrolyzed only by the non-specific (found in serum and plasma) and not by the specific cholinesterase.

Refrigeration and Storage:

Venous blood samples, refrigerated at 5° C. gave consistent cholinesterase values over a period of forty days when the plasma was removed and the cells suspended in a 1:1 physiological saline dilution. Capillary samples can be stored at refrigerator temperature for as long as two months if centrifuged first at 2000 rpm for 20 minutes; otherwise the red cells hemolyze and there is a transition of cholinesterase from the red cell to the plasma or serum fraction.

Flocculation of Red Cell Membrane:

There is a marked decrease in cholinesterase level when the hemolysate is centerfuged for ten minutes at 1200 rpm to remove red cell ghosts. Since the enzyme is bound to the cell membrane and stroma, in flocculation or precipitation, the enzyme activity follows the "ghosts".

Centrifuge Speeds:

Incomplete packing of the red cells by low centrifuge speeds (under 2000 rpm) permits plasma to be trapped with the red cells which produces both a dilution factor and an inclusion of non-specific cholinesterase in the red cell fraction. The subsequent cholinesterase values will be lower than the normal average for the sample.

Stability of Buffer Solutions:

Buffer, three (3) weeks old, even though refrigerated gave values 10% lower than values obtained on the same bloods tested simultaneously with buffer less than two weeks old. Buffers, unrefrigerated, gave cholinesterase values about 50% lower than buffer kept refrigerated until needed.

SUMMARY

1. A technique is described and evaluated for the determination of cholinesterase activity in both red cells and plasma from capillary blood obtained by finger puncture.

2. A comparison of the Michel method with the one herein described shows a significant difference in the averages of the cholinesterase activity observed by the two methods, although there is a high degree of correlation between the methods. Cholinesterase values obtained by the capillary method were higher than those obtained by the Michel method.

3. The capillary sampling method for red cell and plasma cholinesterase on unwashed cells has approximately the same precision as the Michel macro method on washed red cells.

4. The micro test for cholinesterase activity may be used as a screening procedure when successive cholinesterase determinations are desired and when venipunctures are contraindicated. It is also to be recommended for blood sampling when analyses are to be performed at a distant laboratory.

5. It provides a means of blood sampling on small animals for experimental work.

6. Reproducible results can be obtained on individual blood samples allowing for slight day to day variations.

7. Centrifuging the hemolysates before determining the cholinesterase activity precipitates the red cell stroma, giving low cholinesterase values.

8. A decreased 1:1 ratio of saline to red cells will give low cholinesterase values due to the disturbance in ionic equilibrium.

9. Buffers, more than two weeks old, even when refrigerated, decrease in buffer capacity, although the pH may remain only slightly changed.

10. Heparinized venous blood stored for as long as forty days in the 1:1 saline dilution states gives cholinesterase values that are consistent with the original values or fresh blood.

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DETERMINATION OF URINARY 17-KETOSTEROIDS AND 17-HYDROXYCORTICOIDS IN THE CLINICAL LABORATORY*

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There is one great need in most clinical laboratories today, and that is for an endocrine test which can be performed both accurately and rapidly by the medical technologist. Many such tests have been used in research laboratories by specialists highly trained in this type work, but the methods are too complicated for performance in most hospital laboratories. Since unreliable tests are worse than no tests at all, it is necessary to search for reproducible methods which will give clinically accurate results.

Two endocrine tests requested frequently by the practicing physician are 17-ketosteroid and 17-hydroxycorticoid determinations. These findings will give the doctor some knowledge of the adrenocortical function in his patient.

17-Ketosteroids

The endocrine organs, or glands of internal secretion are tissues whose function is to secrete certain specific chemical substances known as hormones into the blood stream, which in turn distributes them to all parts of the body. Certain tissues respond to these hormones in a characteristic way, either increasing, decreasing, or modifying their processes of growth or indicating metabolic or physiological activity. These hormones even may undergo partial or complete oxidation, or reduction transformation. Recognizable products of these transformations, or the hormones themselves, may be excreted in the urine.

Chemically, the known hormones may be divided into two main groups. The first may be referred to as the nitrogenous group. They range in size and complexity from simple bases such as adrenaline, to proteins of high molecular weight of undetermined structure. The second group of a more homogeneous character, may be called the steroid hormones.[1]

The 17-ketosteroids are steroid hormones possessing a ketone group on the seventeenth carbon atom. These may be divided into three general types: acidic, phenolic, and neutral. The neutral are metabolic excretions from the endocrine secretions of the adrenal cortex and the gonads. They may be measured by chemical methods. Clinical manifestations with subsequent fractionation of the 17-ketosteroids are important in the differential diagnosis of various disorders.

The prinicipal 17-ketosteroids are androsterone, isoandrosterone, etiocholanol-3-alpha-17-one, and dehydroisoandrosterone.[2]

Origin of Urinary 17-Ketosteroids

The neutral 17-ketosteroids are produced entirely by the adrenal cortex in the female, and by the cortex and the testes in the male. The

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normal values in a 24-hour urine sample are:*

"Children:	Birth to 3 years	None
	3- 8 years	0 - 2.5 mgm.
	8-12 years	1.5- 5 mgm.
	12-16 years	4 - 9 mgm.
Adult males:		10 -20 mgm. (average 15 mgm.)
Adult females:		5 -15 mgm. (average 10 mgm.)"

Abnormal values may be found in hypofunction of the adrenal cortex or testes. Here the urinary 17-Ketosteroids may be subnormal. Excretion is also low in hypothyroidism, in Simmond's disease, in pituitary tumors, and in brain tumors.

Very high values for 17-ketosteroid excretion have been reported in adrenal hyperfunction, in testicular tumors, and in other conditions such as those caused by emotional and physical strain, by starvation, and by acute illnesses. During the administration of ACTH, the values are also increased.

Modified Kingsley-Getchell Test Tube Extraction Determination of Urinary 17-Ketosteroids

A test tube extraction method has been found which is not considered too difficult for the average clinical laboratory to set up. In the determination, the sample is first subjected to hydrolysis with concentrated hydrochlorid acid since the 17-ketosteroids are present as esters. Extraction with chloroform then follows. The phenolic substances are then removed with 10% sodium hydroxide. This leaves the neutral fraction, which forms colored complexes with the chemical, metadinitrobenzene, the intensity of which color is in the green region of the spectrum and may be measured in the photoelectric colorimeter. Comparison is made with the color developed by known amounts of pure crystalline 17-ketosteroid (dehydroisoandrosterone acetate).[4]

This determination should be carried out in duplicate or triplicate, because only a small aliquot of urine is used and any errors are greatly magnified when converted to a twenty-four (24) hour basis. A mean average of the tests may then be reported.

Urinary 17-Hydroxycorticoids

These steroids have been isolated in crystalline form from cortical extracts. As corticoids they are active in correcting the effects of adrenalectomy. Hydrocortisone, or glucocorticoid is a major corticoid of the blood, a compound derived from the 17-hydroxycorticoid group. It has been found to have quite an influence on protein and carbohydrate metabolism on which its medical uses depend, but this corticoid has only a slight action on sodium and potassium balance. A small amount of the 17-ketosteroid excretion is contributed by the breakdown of 17-hydroxycorticoids. They are not as direct a measurement of adrenocortical activity however, as are the 17-hydroxycorticoids.[2]

* Cantarow and Trumper, *Clinical Biochemistry*, Philadelphia, and London: W. B. Saunders Company, 1949, p. 541.

Metabolism of Adrenal Corticosteroids

"About one-half of the 17-hydroxycorticosteroids are transported in the blood stream where they are loosely bound to the serum proteins. As they pass through the liver, the 17-hydroxycorticoids are conjugated with glucuronic acid and are reduced to the tetrahydro derivatives which are inactive. Both the free and conjugated corticoids are excreted into the intestine by the way of the bile and they are reabsorbed from the intestines by the enterohepatic circulation. Excretion of both free and conjugated corticoids by the kidney take place with a small amount of tubular reabsorption. These steroid hormones combine with the serum albumin causing a low concentration in the urine."[2]

Significance of 17-Hydroxycorticoid

The normal excretion of these corticoids (hydrocortisone) in the urine in adult males is 10 mgm. plus or minus 4; the normal female excretes 7 mgm. plus or minus 3.

A decrease (below 3 mgm. in 24 hours) in the 17-hydroxycorticoid levels may be found in the urine of patients with adrenal insufficiency (Addison's disease), and also with patients having adrenals not stimulated by pituitary, due to the lack of ACTH.[1]

Increased 17-hydroxycorticoid excretion is seen in Cushing's syndrome, in adrenocortical carcinoma as well as in adrenocortical adenoma. The increase is much greater in carcinoma and is not elevated after an intravenous ACTH test as it is in adrenocortical adenoma.

Determination of 17-Hydroxycorticosteroid

The best approach to the study of the adrenal cortex has been found to be the measurement of the levels of 17-hydroxycorticoids in the serum and urine. One of the best methods for diagnosing adrenal insufficiency is in measuring the response of the adrenal cortex after it has been stimulated by the administration of ACTH. An increased level of 17-hydroxycorticoids in the serum and in the urine is found in such cases.

A method that is both simple and rapid is a modified Reddy-Jenkins-Thorn determination. The 17-hydroxycorticoids are excreted primarily as conjugates of glucuronate and sulfate. The urine is acidified with H_2SO_4 to cleave these conjugates, before extraction with butanol. The urine is then saturated with Na_2SO_4 in order to decrease the steroid solubility in the aqueous phase and also to prevent the formation of emulsions. Compounds other than the 17-hydroxycorticoids will be extracted. In treating these extractions with phenylhydrazine a reaction will result which appears to be specific for 17,21-dihydroxy-20-ketosteroids. The mechanism of the reaction has not been explained as yet.[5]

The determination should be carried out in duplicate or triplicate as in the first mentioned test since only a small aliquot of urine is used here, too, for the determination. Errors are greatly magnified when converted to total 24 hour values, and a mean value of several tests should be reported.

Summary

This paper deals principally with a review of the clinical importance

of two very important steroids, 17-ketosteroids and 17-hydroxycorticoids. A summarization of the two tests is given to encourage their use in clinical laboratories. Interesting concepts of the tests are minimized but it is hoped that those interested will further investigate the practical value of these two methods for use in the average clinical laboratory.

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COMPARATIVE STUDIES OF THE CONVENTIONAL WHITE CELL COUNT AND ESTIMATION OF MACRO AND MICRO HEMATOCRIT BUFFY LAYERS*

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Replacement in many laboratories of the RBC by the hematocrit estimation for screening purposes instigated the idea for this study. This procedure has been found to be particularly advantageous when counts are done in a large volume.

Using the preceding statements as a basis, a study on white cell estimation was begun; however, a search for published material on this topic yielded very little information. According to Wintrobe[6] and Ham,[2] a crude estimate of the number of white cells is obtained from inspecting the buffy layer above the packed erthrocytes; this layer consists of packed leukocytes and platelets. In normal blood this layer varies from 0.5 to 1 millimeter, corresponding approximately to 1000 leukocytes per cubic millimeter in the macro-hematocrit.

The number of lymphocytes, monocytes and granulocytes present will influence the thickness of the layer, but for practical purposes cells were counted as leukocytes and not according to classification.

Not only will the type of leukocyte have an effect on the estimation, but the number of platelets will also be a minor contributing factor.[6] In consideration of this fact, their presence was ignored in this study.

The purpose of these studies is to compare the conventional white cell count and the estimation of the count from the macro and micro hematocrit buffy layers. Calculation of the leukocyte count from the buffy layer could be very practical in routine work for screening patients to avoid unnecessary hematological work, since the hematocrit has been shown to be highly reproducible, requires no dilutions, and takes little time to perform.

METHODS

For these studies 2.5 cc. of venous blood was drawn from each patient and added to .25 cc. of dried ammonium and potassium oxalate;[2] this was thoroughly mixed. Oxalate was used as the anticoagulant, since it preserves the cell structure for 24 hours,[3] and these specimens could not always be used immediately. Two leukocyte counts, two macro-hematocrits, and two micro-hematocrits were done on each blood sample, by the following methods:

I. Micro-hematocrit:

Heparinized capillary tubes of soft glass were first used to contain the well mixed blood sample. This type of heparinized tube was employed because it is used routinely in the laboratory; thus it was easily obtained for this procedure. However, the heparin in these tubes was not adequate to prevent the formation in the erythrocyte and buffy layers of small clots which obstructed an accurate reading of the buffy layer. Oxalated blood was, therefore, substituted, which yielded clear individual separation of the layers.

* Scientific Products Foundation Award, Hematology, 3rd. Read before 2th Annual Convention of ASMT, Phoenix, Arizona, June 1959. Received for publication March 1959.

The tubes were filled about ⅔ full, the outside was then wiped clean of blood, and the opposite end was closed to form a flat base by rotating slowly while heating in the periphery of an alcohol burner flame. The filled tubes were centrifuged for 9 minutes at 12,000 revolutions per minute to ensure a compact buffy layer. Less time was found insufficient for complete packing of cells. The capillary tubes were removed and placed upright in a test tube in a rack.

Several methods were tried before one was found that was thought to be satisfactory for an accurate reading. A simple millimeter ruler was used with a hand lens; however, this was very inaccurate because fine enough readings could not be obtained. The indicator scale on an ocular micrometer with divisions to .01 mm. was tried, but this was very complicated for an inexperienced person to use. The procedure found to be the most accurate and practical was by using the Neubauer counting chamber scale on the center large square. The buffy layer was read by placing the tube on the .2 mm. divisions. Using a millimeter ruler and magnification, the total length of the volume in the capillary tube had to be measured, since all the tubes did not contain the same amount. A simple millimeter ruler could be used for this, since the exact total volume was not as important as the precise buffy layer.

II. Macro-hematocrit:

Wintrobe hematocrit tubes were used to contain the oxalated blood. These tubes were filled to the ten centimeter mark with a capillary pipet and then were centrifuged for 30 minutes at 2000 revolutions per minute.* The thickness of the buffy layer was measured with a millimeter scale on the tube with the assistance of magnification with a hand lens.

III. Leukocyte Count:

Standard Exax Pipettes were used to dilute the blood for counting. Using 0.1 N HCl for diluting fluid,[5] a dilution of one to twenty was made. The leukocytes were counted in the four large corner squares on a Neubauer counting chamber. The number of leukocytes per cubic millimeter of whole blood was calculated by multiplying the number of leukocytes counted by 50. Two pipettes were drawn on each specimen, but only one count was done from each pipette, since this is the routine procedure in our laboratory.

IV. Formulas Used:

A. Estimation of count from micro hematocrit[5]

$$\frac{\text{Buffy Thickness in mm.}}{\text{Height of Volume in Tube in mm.}} \times 100 = \%$$

.1 mm. = 1000 WBC ± 20%[6]

B. Estimation of count from macro hematocrit

.1 mm. = 1000 WBC ± 20%[1]

C. Leukocyte Count

No. of cells counted from 4 squares × 50 = Count[4]

MATERIALS

Venous blood from 60 ambulatory patients and hospital patients was used for this study, irrespective of their diagnosis, disease, or treatment. When this study was near completion, it was noted that there were only

* Modified Wintrobe Hematocrit.

a few cases with a white blood cell count above 12,000 and that in these instances the correlation between the hematocrit and actual white count was not the same as it was in counts below this figure; thereafter special efforts were made to collect blood from individuals with known elevated counts.

RESULTS

The sixty patients were divided into four groups according to their leukocyte count as follows: (1) 6,000 and below, (2) 6,000-8,000, (3) 8,000-10,000, and (4) over 10,000. The average actual count, average buffy layers and average estimations were calculated from each person. The maximum error that could be accepted in the estimation was ± 20%. To show the comparison between the estimated counts and actual count, two examples were taken from each group as shown in Table No. 1.

TABLE NO. 1
Comparison Between Estimated Counts and Actual Counts With the Percentage of Error by Estimation

	Actual Count (Average)	Buffy Layer Ave.	Micro Estimation	Percent Error*	Macro Buffy Layer	Estimation by Macro	Percent Error*
I.	5,675.....	.53 mm.	5,300	+7%	.5 mm.	5,000	+13.5%
	5,925.....	.72 mm.	7,200	—17.7%	.625 mm.	6,250	—5.2%
II.	7,050.....	.68 mm.	6,800	+3.2%	.75 mm.	7,500	—6.0%
	7,375.....	.835 mm.	8,350	—11.7%	.75 mm.	7,500	—1.5%
III.	8,975.....	.935 mm.	9,350	—4%	.875 mm.	8,750	+2.6%
	9,450.....	.905 mm.	9,050	+4.4%	1 mm.	10,000	—5.5%
IV.	10,075.....	.975 mm.	9,750	+3%	1 mm.	10,000	+7.5%
	16,475.....	1.445 mm.	14,450	+14%	1.5 mm.	15,000	+9.8%

* Refers to difference between actual count and estimated count.

In the three classes under 10,000 the average buffy layer was calculated for each group according to micro or macro hematocrit and the average estimated count with the per cent of error of the specific method as shown in Table No. 2. The counts in the last group were not evenly distributed between 12,000 and 17,000; therefore, it was impossible to get an accurate average of the buffy layers, estimated counts, or per cent of error.

As can be seen from the following averages, as long as the count is under 12,000 the estimation is fairly accurate by both hematocrit methods, but as the count increases the hematocrits are less accurate. Over 12,000 the micro-hematocrit error increases to 39%, while that of the macro is

TABLE NO. 2
Comparison Between the Average of the Counts and the Estimation With the Percentage of Error

Group	Ave. Calculated Buffy Layer	Estimated Count Micro	Percent Error*	Ave. Buffy Layer-Macro	Estimated Count Macro	Percent Error*
4,000-6,000 (Ave. from 6 counts)	.586 mm.	5,860	—14.7%	.6875	6,875	—27.3%
6,000-8,000 (Ave. from 20 counts)	.702 mm.	7,020	—.3%	.656 mm.	6,560	+ 6.7%
8,000-10,000 (Ave. from 18 counts)	.7497 mm.	7,497	+20 %	.8736 mm.	8,736	+ 3 %
10,-12,000 (Ave. from 8 counts)	.9325 mm.	9,325	+17.9%	.935 mm.	9,350	+17.6%

* Refers to difference between actual count and estimated count.

not over 27% with the exception of a 60% and 77% error in two separate instances, as shown in Table No. 3.

TABLE NO. 3

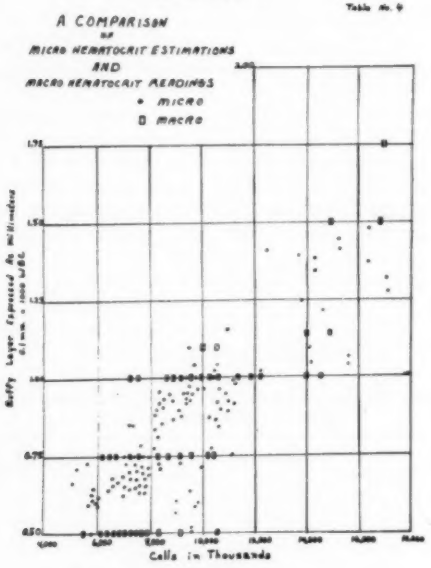
Comparison of the Estimated Counts and Actual Counts With the Percentage of Error in Estimating Counts Over 12,000

Actual Count Ave.	Buffy Layer Micro-Ave.	Estimation of Count	Percent of Error*	Buffy Layer Macro-Ave.	Estimation of Count	Percent of Error*
12,125	.98 mm.	9,800	+23.7%	.75 mm.	7,500	+61.6%
13,925	1.33 mm.	13,300	+ 4.7%	1.5 mm.	15,000	— 7.2%
14,025	1.075 mm.	10,750	+30.0%	1.1 mm.	11,000	+27.5%
14,275	1.3775 mm.	13,775	+ 3.6%	1.5 mm.	15,000	— 6.2%
14,675	1.19 mm.	11,900	+23.3%	1.35 mm.	13,500	+ 8.7%
15,000	1.455 mm.	14,550	+ 3.0%	1.5 mm.	15,000	0 %
15,525	1.12 mm.	11,200	+38.6%	.875 mm.	8,750	+77.0%
16,475	1.445 mm.	14,450	+14.0%	1.5 mm.	15,000	+ 9.8%
16,950	1.275 mm.	12,750	+32.9%	1.675 mm.	16,750	+1.19%

* Refers to difference between actual count and estimated count.

It will be noted from the accompanying diagram, the close proximity of estimated readings made from micro-hematocrit as compared with those of the macro-hematocrit. This diagram also shows the change in direction of the scatter pattern at an elevation above 12,000 WBC., as shown in Table No. 4.

TABLE NO. 4



COMMENTS

It is this investigator's opinion that the estimation of a white count from the buffy layer of a hematocrit would not far exceed ± 20% of the actual count, if it is normal. It is probable that the micro-hematocrit

estimation is more accurate from this limited survey for this procedure than the macro method. As contrasted with the macro-hematocrit, the micro-hematocrit gives a more reliable reading because of greater magnification, sharper demarcation between the layers, and the use of a more precise scale.

However, the use of this estimation is not suggested for its accuracy alone; therefore, both hematocrit methods could be used by the physician for screening the white cell count of patients. For practical purposes this procedure is recommended to be used to decide whether a patient has a normal or elevated leukocyte count. For a precise white cell count the use of the pipet and counting chamber cannot be impugned; however, if a screening were done it would require fewer white counts and enable the technologist to devote his time to other work.

It is quite evident that such a method would save a great amount of time. The greatest disadvantage is the fact that venous blood, well-oxalated, must be used. An oxalated capillary tube may be the answer; however, time did not permit further study in this direction.

SUMMARY

Blood from sixty patients was drawn and a comparison was made between the leukocyte count and the estimation of the count from the buffy layer of the macro and micro-hematocrit. It was found that the estimated count, as a rule, is accurate within ± 20% of the actual count, if the count is less than 12,000. From this standpoint such a procedure could be used for screening patients to avoid excessive hematological work. It was also discovered that the micro-hematocrit is much simpler and easier to use than the macro-hematocrit; it also can give a more accurate reading requiring less blood.

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DISTINGUISHING CHARACTERISTICS OF AUTOHEMAGGLUTININS AND ROULEAUX PHENOMENA*

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INTRODUCTION

Cold agglutinin, auto-antibody and rouleaux phenomena are not in themselves a contraindication for transfusion. However, their presence can obscure the correct blood group and Rh type of the person, as well as render the crossmatching tests difficult to evaluate. It is, therefore, extremely important to be aware of their presence and to be able to distinguish one from the other so that proper technics, designed to cope with the unique properties of each one, will be used.

RECOGNITION

Suspect the presence of one of the above conditions when blood grouping tests show "AB," the reverse grouping "O," and all Rh typings are positive. The routine use of "negative controls" in all grouping and typing procedures will reveal the presence of any of these three conditions, since the abnormality lies in the serum of the person. Therefore, always include as one negative control, the serum of the person being studied. The use of 30% bovine albumin as a negative control will also detect autohemagglutinins and should be included in the test panel. An example of such a test panel is illustrated in Table I.

TABLE I

Example of Grouping and Typing Using Controls:

Blood Sample		SPECIFIC ANTI-SERUMS				NEGATIVE CONTROLS		
		A	B	D	CDE	Serum of Blood Being Tested	Bovine Albumin 30 Percent	
Normal Blood	"O", D +	—	—	+	+	—	—	
	"O", D —	—	—	—	—	—	—	
Cold Agglutinin	"O", D +	+	+	+	+	+	+	*
	"O", D —	+	+	+	+	+	+	
Auto-Antibody	"O", D +	+	+	+	+	+	+	*
	"O", D —	+	+	+	+	+	+	
Strong Rouleaux	"O", D +	+	+	+	+	+	+	*
	"O", D —	+	+	+	+	+	+	

* The intensity of the non-specific agglutination will vary with the titer of the autohemagglutinin and will also depend upon whether it is accompanied by specific agglutination or not.

IDENTIFICATION

The following key[1] will quickly indicate which of the three conditions is present in a serum. The key is based on the performance of three simple test procedures. These tests are as follows:

1. A slide agglutination test, using the persons own serum and red cells in the proportion of one drop of serum to two drops of whole blood. The slide containing this mixture is then placed on a view box with a surface temperature between 40 and 45° C. and observed for a two minute period.

* Read on the program of 27th Annual Convention of ASMT, Phoenix, Arizona, June 1959. Received for publication February 1959.

2. Elution of the patients' red cells with physiological saline performed both at 25° C. and 37° C.
3. The Direct Coombs test.

KEY TO THE IDENTIFICATION OF THE AUTOHEMAGGLUTININ

A. Red cells agglutinated by own serum.
I. Agglutination weakened or reversed by heat (40° C.) on slide.
a. Red cells washed* (eluted) with warm saline solution (37° C.).
a.a. Direct Coombs test NEGATIVE
∴ COLD AGGLUTININ
a.b. Direct Coombs test POSTIVE
∴ COLD AGGLUTININ + INCOMPLETE COLD AGGLUTININ AND/OR AUTO-ANTIBODY
II. Agglutination unchanged or strengthened by heat (40° C.) on slide.
a. Red cells washed* (eluted) with cold saline (20-25° C.).
a.a. Direct Coombs NEGATIVE
∴ ROULEAUX PHENOMENA
a.b. Direct Coombs POSITIVE
a.b.c. Red cells washed* (eluted) with warm saline (37° C.).
1. Direct Coombs NEGATIVE
∴ COLD AGGLUTININ
2. Direct Coombs POSITIVE
∴ AUTO-ANTIBODY AND/OR INCOMPLETE COLD AGGLUTININ

DISCUSSION

Rouleaux Phenomena

Definition: Rouleaux phenomena refers to the formation of red cells into rows, similar in appearance to stacks of coins that have been tipped over. The cause of this phenomena is the elevation of macro-globulins and fibrinogen in the serum or plasma and is not a defect of the red cells.

Clinical Characteristics: It is encountered in illnesses where the sedimentation rate is elevated because of alteration in the globulins. For this reason, it is more frequently encountered in the patient's serum than in the donor's, thereby causing difficulties in the major crossmatch.[2,5]

Technical Characteristics: The heat used in Rh typing and crossmatching procedure, using the slide technic, increases the formation of rouleaux because of the concentrating effect of evaporation. This is first noted at the edge of the preparation and is quickly dispersed by one or two drops of saline without interfering with any specific agglutination[9] that may be present. Unless strong rouleaux is present, microscopic examination under a cover-slip will verify true agglutination.

Tube technics eliminate the concentration effect due to evaporation but centrifugation may pack rouleaux into masses similar in appearance to true agglutination.

Verification of Blood Group and Rh Type: True agglutination of the cells being tested can be obtained by washing the cells free of serum or plasma and resuspending to a 40% concentration of red cells in 6% bovine albumin for slide tests, or to a 2% concentration in saline for

* Cell washing (eluting) technic: Red cells should be washed four times with 10 ml. volumes of physiological saline, completely resuspending the cells each time. Sometimes cold agglutinin and even rouleaux phenomena occur in such high titer, that it is necessary to wash the cells many times in order to remove them completely. In rare cases, cold agglutinins cannot be removed and are indistinguishable from auto-antibody.

tube tests. Use these cells for rechecking the blood group and Rh type.

Confirmation of Blood Group and Crossmatch: Since the rouleaux forming properties cannot be removed from the serum,[10] the indirect Coombs crossmatch *may* be the only way of demonstrating the presence or absence of incompatibilities. The presence of strong Rouleaux in the serum makes confirmation of blood group by back grouping unreliable.

Cold Agglutinin

Definition: Cold agglutinins are autohemagglutinins whose thermal optimum is between 0 and 10° C. Autohemagglutinins are antibodies that agglutinate the cells of the individual from whom the serum originates, as well as all other human red cells.[3, 6]

Clinical Characteristics: They may be associated with the patient's clinical condition but they also occur in apparently normal healthy subjects.[11]

Technical Characteristics: Usually agglutination by cold agglutinins is completely reversible at temperatures between 20 and 37° C. However, high titer agglutinins may persist at 37° C. or higher and may be so firmly affixed to the red cell (incomplete antibody) that it will not readily elute from the cell. This is demonstrated by a positive direct Coombs test.

Verification of Blood Group and Rh Type: True agglutination of the cells being tested usually can be demonstrated after repeated washing in warm saline (37° C.) until the cells no longer agglutinate in 30% bovine albumin at 20° C. Use these cells for verifying blood group and Rh type.

Confirmation of Blood Group and Crossmatch: The serum can usually be freed of cold agglutinins by absorption with its own red cells as follows:[12]

1—Obtain two blood specimens from the patient, one oxalated and one clotted. Allow specimen for serum to clot in refrigerator (4-10° C.). Keep this tube cold (use iced centrifuge cups) during centrifugation and remove serum immediately after.

2—Keep oxalated tube warm (37° C.) and wash cells twice with warm saline.

3—To absorb serum, use equal quantities of serum and washed, packed cells and place in refrigerator for 15 minutes.

4—Centrifuge in iced cups and remove serum and check for complete removal of cold agglutinin by testing with a 2% suspension of the person's own washed cells.

5—If it is necessary to reabsorb serum, elute the antibody from the cells used in the first absorption with warm saline in a waterbath at 37° C. for 15 minutes. Centrifuge in warm cups, discarding the supernatant saline and using the cells to absorb the serum again as in steps 3 and 4.

6—Steps 3, 4 and 5 should be repeated until all antibody has been absorbed.

7—Use this absorbed serum for crossmatching procedures and for confirming the ABO group by reverse typing.

Auto-Antibody

Definition: Auto-antibody is an autohemagglutinin of the warm variety

whose thermal optimum is 37° C.[6, 7]

Clinical Characteristics: This is an example of true auto-immunization whereby the individual is immunized to antigens present in his own tissues, resulting in sensitized (coated) cells as in patients suffering from acquired hemolytic anemia. A child suffering from hemolytic disease of the newborn will have similarly coated cells.[8] Some times at birth the red cells are blocked (coated) by absorbed maternal antibodies, the antigen receptors on the cell surface being saturated with incomplete antibody.

Technical Characteristics: Their presence gives the red cells a positive direct Coombs reaction and sometimes may cause agglutination in albumin alone. It is not clinically practical to absorb auto-antibody from the serum nor can the coated cells be freed of antibody without some destruction of the stroma of the cell. For these reasons the presence of auto-antibody presents a transfusion dilemma, since high protein and indirect Coombs crossmatching are invariably positive with all donor cells. This makes a potentially dangerous situation since patients suffering with acquired hemolytic anemia receive multiple transfusions and may be immunized to specific antibodies as well.

Verification of Blood Group and Rh Type: Wash coated cells free of serum or plasma and perform grouping and typing tests using 30% bovine albumin as a negative control. Non-specific agglutination (albumin alone) may be less intense than the specific agglutination with the test anti-serums. In this way an indication of the correct group and type may be obtained.

Confirmation of the Blood Group and Crossmatch: Since an uncertainty exists concerning the correct group and type, and because the patient needs blood, perhaps the safest procedure is to use the blood of a donor as close to the group and type of the patient as it is possible to ascertain. Comparison crossmatching should be done, using the patient's serum with his own cells as a negative control. If the degree of clumping with the donor's cells is no greater than with the patient's cells report it provisionally compatible, with reservations. EXTREME CARE MUST BE EXERCISED in transfusing this type patient, a physician should be present and the transfusion stopped at the slightest sign of reaction.

Since auto-antibody cannot be absorbed from serum, confirmation of blood by back typing is impossible.

SUMMARY

A simplified key is presented with the purpose of providing a means for quickly recognizing the presence of, and distinguishing, cold agglutinin, auto-antibody and rouleaux phenomena one from the other. A brief discussion is given, emphasizing the need for special methods of grouping, typing and crossmatching in each instance.

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STUDIES OF STREPTOBACILLUS MONILIFORMIS FROM A CASE OF HUMAN RAT-BITE FEVER**

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It was stated by Dubos[2] that a natural body fluid, such as ascitic fluid or blood serum, is needed for the growth of Streptobacillus moniliformis on artificial media. It was later shown that the serum component could be replaced by starch,[3] glycogen or dextrin.[4] A case of human rat-bite fever afforded our laboratory the chance to study the effects of ascitic fluid and blood serum as enrichments when combined with these base media; trypticase soy broth and agar, tryptose phosphate agar and dextrose starch agar. Biochemical and sensitivity studies were also investigated.

Materials and Methods

The patient's blood was inoculated into two flasks of trypticase soy broth* containing 0.1% agar. Because rat-bite fever was suspected by the clinician, one culture was placed under 8% CO_2 tension at 37° C. after incubation at 37° C. for 12 hours.

After 72 hours incubation, organisms resembling Streptobacillus moniliformis were seen on smears taken from the culture incubated under CO_2 tension. Duplicate subcultures from this specimen were plated on dextrose starch agar, tryptose phosphate agar and trypticase soy agar,* enriched with 20% ascitic fluid or horse serum. All subsequent subcultures, biochemical and sensitivity studies were incubated at 37° C. under 8% CO_2

Agar "cut outs" from the above plates were transferred to trypticase soy broth and tryptose phosphate broth. Both of these were run in duplicate and enriched with ascitic fluid or horse serum. Rabbit serum was used in later subcultures.

Sensitivity studies were carried out by the tube-serial dilution method using enriched trypticase soy broth and incubated for 24 hours.

A base medium of cystine trypticase agar,* 10% horse serum and 1% of the various carbohydrates was used in fermentation studies and incubated for 96 hours.

Results

The blood culture incubated under 8% CO_2 tension for 72 hours showed wavy pleomorphic rods on Gram's and Giemsa's stained smears. The blood culture incubated without increased CO_2 tension was sterile after fifteen days.

Subcultures on the enriched dextrose starch agar, tryptose phosphate agar and trypticase soy agar, after 72 hours incubation, showed a sparse growth of small, white, smooth, raised colonies. There was no difference in the number or appearance of the colonies on the dextrose starch or trypticase agar containing either enrichment. Fewer colonies appeared on tryptose phosphate agar with either enrichment. Gram's and Giemsa's

* Products of Baltimore Biological Laboratories, Inc.
** Received for publication July 1959.

stained smears from the colonies revealed wavy, filamentous rods with deeply stained, spindle shaped swellings. A few coccobacillary forms were noted (Figure 1).

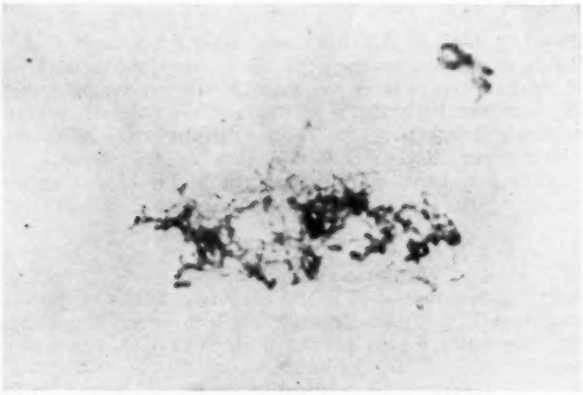

Figure 1. Smear of Streptobacillus moniliformis showing the characteristic wavy filamentous rods with spindle shaped swellings and a few coccobacillary forms. X 970.

The two enriched broth media inoculated with agar "cut outs" showed characteristic "fluff balls." The density of the growth was greater in the trypticase soy broth enriched with horse or rabbit sera. Tryptose phosphate broth with either enrichment supported growth poorly. The "fluff ball" growth appeared in broth after 48 hours beginning 1.5 cm. below the surface and extending to the bottom of the tube (Figure 2). Organisms were not identified, by smear, in the surface area.

Biochemical and sensitivity studies of Streptobacillus moniliformis are shown in tables I and II respectively.

Discussion

These studies showed that trypticase soy agar and broth and dextrose starch agar enriched with either 20% horse serum or rabbit serum were the best of the media, which we used, for supporting the growth of human strains of Streptobacillus moniliformis. These findings correspond with those of previous workers. Tryptose phosphate agar supported growth very poorly, an observation which disagrees with earlier reports.[2] A stock broth culture can be maintained by freezing and transferring every six weeks. The biochemical studies are essentially the same as those given in the 7th edition of Bergey's Manual of Determinative Bacteriology.[1]

Antibiotic sensitivities showed that the organism is resistant to chloromycetin in a dilution of more than 50 units per cc. The organism is more sensitive to the other antibiotics studied.

Figure II. Broth showing the characteristic "fluff ball" growth of streptobacillus moniliformis.

TABLE I
Biochemical Studies

Biochemical Test	Reaction	Biochemical Test	Reaction
Glucose	A	Inulin	NC
Lactose	NC	Dulcitol	NC
Sucrose	NC	Inositol	NC
Maltose	A	Raffinose	NC
Mannitol	NC	Sorbitol	NC
Salicin	NC	Trehalose	NC
Dextrin	A	Rhamnose	NC
Levulose	A	Adonitol	NC
Galactose	A	H_2S	Slightly positive
Glycerol	NC	Indole	Negative
Xylose	A	.....	....

Acid—A
No Change—NC

TABLE II
Antibiotic Sensitivity Studies

Antibiotic	Minimal Inhibiting Concentration	Antibiotic	Minimal Inhibiting Concentration
Penicillin	Less than 0.17 U/cc	Chloromycetin	50 Mcg/cc
Streptomycin	3.1 Mcg./cc	Aureomycin	0.77 Mcg/cc
Terramycin	3.1 Mcg/cc	Polymyxin B	Greater than 50 U/cc
Tetracycline	Less than 0.17 Mcg/cc	.....	.....

Micrograms—Mcg
Units—U

Summary

Streptobacillus moniliformis was isolated from a case of human rat-bite fever. Cultural studies showed that trypticase soy broth or agar and dextrose starch agar enriched with horse or rabbit sera were the best media in our laboratory for sustaining the growth of the organism, whereas tryptose phosphate agar supported growth poorly. We found that the organism must be incubated at least 72 hours at 37° C. under 8% CO_2 tension for growth and isolation.

Biochemical and antibiotic sensitivity studies are presented.

Acknowledgments

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DETERMINATION OF COPPER AND IRON*

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The compound, 1, 10-phenathroline, and its substituted derivatives have been employed in many instances for the determination of copper and iron in various media. A recent communication from this laboratory[1] has described the application of the most sensitive of these compounds to small samples of blood serum. The color-forming reagents were bathophenanthroline (4,7-diphenyl-1,10-phenanthroline) and bathocuproine (4,7-diphenyl-2,9-dimethyl-1,10-phenanthroline) for iron and copper respectively. These were employed in their sulfonated, water-soluble forms, a modification which was first suggested for bathophenanthroline by Trinder.[4]

Because of the possibility that investigators working with a variety of other biological and non-biological materials might find the described serum technic unsuitable for their purposes, several modifications have been devised to extend the range of usefulness of this method of trace metal analysis. The modified procedures, which form the basis of this report, are of two types: (1) the use of a two-phase system, in which one of the metals is determined in aqueous solution by means of the appropriate color reagent in its sulfonated, water-soluble form, while the other metal is subsequently extracted from the same aliquot into an organic solvent containing the second color reagent in the unmodified, water-insoluble form; and (2) the simultaneous analysis of both metals in either the aqueous or the organic phase. The previously described method of analysis of separate aliquots in the aqueous phase, which we consider the simplest and most suitable procedure for routine serum determinations, was carried out simultaneously with the modified methods in this study, and is presented as technic "A," below. Because copper and iron analysis of biological materials usually requires leaching of the metals from their binding sites and subsequent precipitation of the organic matter, these preliminary steps are also described. A slight modification of the original technic will be noted, in that acidification of the matrix is carried out with 3N hydrochloric acid, rather than 1N. This modification was prompted by reports stating that the lower acid concentrations are insufficient to liberate the metal ions from their protein bonds.[3] (Actually, in our experience, results are identical with either acid concentration.)

Methods

Reagents

1. Color reagents for organic phase. Prepare separate solutions of bathophenanthroline and bathocuproine in iso-octyl alcohol, each in a concentration of 30 mg. per 100 ml.
2. Sulfonated iron color reagent: Dissolve 50 mg. of bathophenanthroline

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sulfonate* in 10 ml. of metal-free distilled water in a 100 ml. volumetric flash. Dilute to the mark with a saturated solution of sodium acetate with vigorous mixing. Warm to effect solution and filter if necessary after cooling.

3. Sulfonated copper color reagent: Heat 100 mg. of bathocuproine with 0.5 ml. of chlorosulfonic acid over the flame of a microburner for 30 seconds. Cool, and carefully add 10 ml. of metal-free distilled water with mixing and subsequent warming in a water bath to facilitate solution. Dilute 3.0 ml. of this solution to 100 ml. with a saturated solution of sodium acetate, warm to effect solution, cool and filter if necessary. (The sulfonated form of bathocuproine is currently available commercially.*)

4. Copper and iron working standard solutions: Pipet 0.0, 1.0, 2.0 and 3.0 ml. of either a copper or iron stock standard solution (50 mg. per liter) into separate 100 ml. volumetric flasks. Add 25 ml. of 3N hydrochloric acid and 25 ml. of 10% trichloracetic acid to each and dilute to the mark with metal-free distilled water. (The acid concentrations of these solutions correspond to those of filtrates of biological materials prepared according to the leaching-precipitating step described below).

Procedures

Leaching-precipitating method for serum: Pipet 2 parts of serum and 1 part of 3N hydrochloric acid into a centrifuge tube, mix well, heat the mixture for 5 minutes in a boiling water bath and then cool it. Add one part of 10% trichloracetic acid, mix well, and centrifuge at 4000 r.p.m. for 10 minutes.

Technic A: Pipet two 0.5 ml. aliquots of the clear supernate into separate micro cuvets and add a few milligrams of solid ascorbic acid. Add 150 microliters of the sulfonated color reagents, mix well, and read the absorbances at 485 mu for copper and 535 mu for iron against appropriately prepared blanks. In the event that a filtrate is not completely colorless, add 150 microliters of saturated sodium acetate solution containing no color reagent to a third aliquot of filtrate and read it against a similarly prepared blank for extraneous color correction. (Because of the yellow color involved, this is a more important consideration for the copper determination than it is for the iron determination.)

Technic B: Pipet 1.0 ml. of either sulfonated color reagent into a 4.0 ml. aliquot of filtrate. Add a small spatula tipful of solid ascorbic acid, mix, and read the absorbance at the proper wave length against a similarly prepared blank (we have used a DU Beckman spectrophotometer with test-tube adapter assembly). Add 5.0 ml. of an iso-octyl alcohol solution of the other color reagent and extract vigorously for 5 minutes. Centrifuge to separate the layers, remove the lower aqueous layer with a long needle and syringe, and centrifuge again for 2 minutes. Read the absorbance of this sample against its blank at the appropriate wavelength.

Technic C: Pipet 0.5 ml. of each sulfonated reagent into 4.0 ml. of filtrate and add solid ascorbic acid. Mix and read the absorbances at

* Available from G. Frederick Smith Chemical Company, Columbus, Ohio.

485 mu and 535 mu against a blank containing both color reagents. Alternatively, extract into an iso-octyl alcohol solution of both color reagents (30 mg. per 100 ml. of each) after adding solid ascorbic acid to the aliquot of filtrate, discard the aqueous layer after centrifugation, and read the absorbances at both wavelengths. The concentrations of each metal may then be determined by the application of simultaneous equations,[2] the use of which requires preparation of calibration curves for each metal at both wavelengths and a monochromatic spectrophotometer such as the DU Beckman to achieve maximum resolution of the spectra for employment in simultaneous equations.

Discussion

Control of pH and provision of an excess of color reagent for the amount of metallic ion present in samples are the major factors necessary to insure the success of these procedures. These considerations have been discussed in some detail in previous publications,[1,5] and it has been shown that the employed proportions of sodium acetate solution and of color reagent do, in fact, meet these requirements.

The leaching and precipitating manipulations described for serum samples differ in no important respect from those investigated and described by many other workers, and now accepted as adequate for these purposes.[3] It may be added that we have found heating not absolutely necessary, if acidified serum samples, 1N in hydrochloric acid, are allowed to stand at room temperature for 30-60 minutes before adding the protein precipitant; however, high centrifugation speeds are necessary to obtain a clear filtrate under these circumstances.

A comparison of the analytical results obtained with the three proposed technics is shown in tables 1 and 2. Table 1 shows the results of determinations of copper and iron content of aqueous solutions of varying concentrations; table 2 demonstrates the application of these technics to a number of samples of pooled serum. In both, there is good

TABLE I

Determination of Known Amounts of Copper and Iron From Mixtures Using Several Technics

	Micrograms Present					
Technic Used	Cu. Pres.	Fe. Pres.	Cu. Fnd.	Fe. Fnd.	Cu. Diff.	Fe. Diff.
A	0.50	3.00	0.60	2.95	+0.10	—0.05
A	1.00	3.00	1.07	3.00	+0.07	0.00
A	2.00	3.00	2.10	2.90	+0.10	—0.10
A	3.00	1.00	2.95	1.05	—0.05	—0.05
A	3.00	0.50	2.95	0.50	—0.05	0.00
B	1.00	3.00	1.04	2.90	+0.04	—0.10
B	2.00	3.00	2.06	3.10	+0.06	+0.10
B	3.00	3.00	3.02	2.95	+0.02	—0.05
B	3.00	2.00	2.99	1.90	—0.01	—0.10
B	3.00	1.00	2.95	0.98	—0.05	—0.02
C	1.50	3.00	1.60	2.92	+0.10	—0.08
C	0.50	3.00	0.57	2.90	+0.07	—0.10
C	3.00	3.00	3.00	3.10	0.00	+0.10
C	1.00	3.00	0.90	3.10	—0.10	+0.10
C	2.00	3.00	1.90	3.10	—0.10	+0.10
C	3.00	3.00	2.80	3.06	—0.20	+0.06
C	3.00	2.00	2.90	2.06	—0.10	+0.06
C	3.00	1.00	2.70	1.07	—0.30	+0.07

TABLE II

Comparison of Several Technics for the Determination of Copper and Iron in Aliquots of Several Filtrates of Pooled Serums

Mcg. Per 100 ml. Found					
TECHNIC A		TECHNIC B		TECHNIC C	
Cu. Fnd.	Fe. Fnd.	Cu. Fnd.	Fe. Fnd.	Cu. Fnd.	Fe. Fnd.
174	65	167	59	155	63
151	56	153	52	157	54
170	83	175	83	168	86
188	70	183	66	178	68
184	91	179	89	185	90
183	230	185	239	175	243
198	146	200	136	188	144
168	57	173	54	158	56
163	171	158	175	167	174
215	63	213	62	220	65

agreement of values obtained with different methods for absolute amounts in assorted mixtures of the metals as well as comparative recoveries from a number of filtrates.

Summary

The high sensitivity and specificity of bathophenanthroline and bathocuproine as color complexing reagents for iron and copper respectively, make these compounds the best currently available for the quantitative analysis of trace amounts of these metals. The ability of these water-insoluble compounds to acquire aqueous solubility after sulfonation without loss of other properties, has made it possible to devise several simple and accurate analytical technics which may be applied to the determination of copper and iron in a variety of circumstances and in both biological and non-biological media.

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MOTIVATION FOR THE USE OF CONTROLS IN THE LABORATORY AND PARTICIPATION IN EXPERIMENTS DESIGNED TO DISCOVER SOURCES OF ERROR*

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Introduction

This paper represents an attempted summary and review of some of the author's remarks in a joint presentation of a Continuation Course in Medical Technology for Technologists. The course was offered at the Center for Continuation Study, University of Minnesota.

The early part of the course was confined to simple concepts in statistical methodology and their application to laboratory problems. Measures of location and variation were discussed. Frequency distributions in general, and the normal curve were discussed. A brief introduction to quality control methods was attempted. Since the material covered during the early part of the session is readily available in elementary text books,[1,7,14] Quality Control text books,[5,9] and several articles in the bio-medical journals,[10,11,12,13] this summary will not deal further with that material.

The text that follows deals only with an attempt to motivate more complete utilization of available statistical tools in the laboratory. In preparation for the course it was somewhat of surprise to find that we were not breaking new ground in any sense of the word. Most of the pertinent points have been discussed very well by persons in your own field in your own journals. The tools are available. At least some of you are aware of their value. Apparently all that is needed is broader motivation to break the inertia.

I. The Desirability of a General Awareness of the Error Associated with A Specific Laboratory Determination

A system of Quality Control or a specific study of components of variance will, in general be designed to determine the size of the error, its components and to find ways of reducing the error. It is possible, however, that the most important consequences of the experiment or the installation of the Quality Control program will be forthcoming long before the analysis of the completed available data takes place. These important consequences have to do with the fact that most of the members of the laboratory team, and the clinicians utilizing the services of the laboratory, will become error-conscious. The error distribution will come to light. When the final analysis is completed, you may be satisfied with the status quo, that is, the errors may be small enough to be tolerated for the purposes of the determination. You may wish to improve. These things will depend upon the ultimate results of the experiment. Whatever your final conclusions you will know the size of the error. You will continue to attempt to get measurements on the size of the error and as a team you will continue to be aware of the

* With only slight modifications, this is essentially a reprint of a paper of the same title published earlier; (The Minnesota Medical Technologist, Jan. 1957).

sources of error. This thought will be emphasized throughout the remainder of the discussion. Some basic reasons for focusing attention on error, reasons other than a routine attempt to reduce error, are listed below.

A. Some determinations are fraught with error and not subject to improvement. At least no reasonable improvement can be expected within the economical limitations of the laboratory. Such determinations might actually be dropped if those responsible were aware of this critical situation. A good example of this type situation might be had in the red-cell counting procedure. We realize that the accuracy of red-cell counting is difficult to obtain and a substitute measure, the hematocrit determination has been pretty well accepted by the clinicians.

B. Unfortunately, many clinicians are not aware of the variability associated with chemical determinations. Please do not take this as direct criticism of the doctors involved. The fault often lies with you, the laboratory technologists. You may have been responsible for some of his training in the beginning and you are certainly responsible for his continued ignorance of the variability of the determination in that you have not pointed out the variation in his daily contact with your laboratory. In this connection I would like to emphasize that the material available in journals concerning variability of determinations obtained by certain specific investigators on special experiments cannot be transferred to your laboratory experience. What the doctor in your hospital wants is the error distribution for your laboratory, for your equipment and for your personnel.

C. Increased cooperation and education may lead to a statement of desired precision by clinicians or by investigators who are utilizing the services of the laboratory. The method presently being used may be unnecessarily accurate, unnecessarily difficult, and unnecessarily expensive. The method being used may not be even close to the desired accuracy. Under the existing conditions of personnel, equipment, and overwork, the desired accuracy may not be obtainable. Usually the only time that financial or personnel assistance are forthcoming for the laboratory is when the administrators discover that their laboratory is too slow. That is, they don't turn out a sufficient amount of routine work within a specified time. Focusing attention on the necessity for increased accuracy may make the administration more realistic in the choice of a measuring stick as to the efficiency and value of the clinical laboratory. In other words, more money would be allocated to bring about increased accuracy, not increased speed.

D. This item should probably be considered a part of the above, but is being considered separately. It is actually possible to look upon any given determination from two points of view. The doctor may be following a medical patient with extremely close supervision. In this case his determinations from the laboratory should be as accurate as possible. Conversely, the doctor may be sending in samples from routine checkups, periodical physicals, etc. and require a simple statement from the laboratory that the values determined are within normal bounds. It stands to reason that the screening procedure designed simply to tell whether the unknown value being determined is above or below a certain limit

would be easier, less expensive and much faster than an attempt to get accurate determinations. Unfortunately, the doctor usually does not inform the laboratory as to what the purposes are for the determination that he is requesting. The money, time and effort saved in a quick screening determination could be applied toward getting more accuracy in the other situations where extreme accuracy is desired and necessary. This sort of attitude has been adopted by industry in several situations. Health departments, in determining coliform counts in water sources, rather than estimate the density, simply determine whether the count is bigger than a certain specified critical value. Personal observation of behavior of doctors and questioning several whose opinions are widely respected would indicate that a large share of the routine determinations presented to the laboratory could be viewed in this light. The benefits to be gained are obvious but a very significant change in attitude such as this can take place only as a result of education and cooperation. The entire hospital staff has to be made aware of the error distribution and its significance. Examples of screening techniques are given in reference 9.

II. Some General Benefits To Be Derived From A Quality Control Program

A. For emphasis we reiterate. The simple concept of continual awareness of and reporting of the magnitude of error which will result from a quality control program will probably be the most important significant benefit derived therefrom.

B. A chronological log book wherein all significant changes, adjustments and happenings in the laboratory are recorded by hour and date is an essential part of the control program. This log book when analyzed in conjunction with the Quality Control charts on location and variation will pinpoint sources of error not otherwise discernible by routine application of administrative supervision in the laboratory.[12]

(1) Slow trends, such as deterioration of reagents or batteries which take place over several days will become apparent only when the trends are observed on the control chart and seem to stop or readjust when the reagent or batteries are changed.

(2) Cyclical trends come to light by analysis of the material in the log book and the cycles of the observations on the control chart.[12] Certain laboratory days have heavier loads and therefore the number of determinations handled in a given run on a particular day may be larger than on another day. This may contribute to a larger variability on the overworked days. Line voltage may vary from hour to hour and a daily 24-hour cycle may therefore be seen on determinations depending upon electrical equipment.

(3) Big, somewhat unexplainable or apparently random jumps in the Quality Control charts often will be found to correspond to changes in equipment or personnel or material.[10, 13]

(4) The accuracy of the determination often hinges upon extraneous variables such as temperature or reaction time. A careful and complete record in the log of various temperature changes, the length of time that material has been standing between readings, etc., will possibly lead to the discovery of the optimum temperature reaction time relationship

for giving maximum accuracy. This sort of information can be obtained only by long-term experimentation and careful record keeping.

(5) Accuracy often depends upon the magnitude of the expected unknown determinations.[5, 6] For instance, in cell-counting the less dense the cells in the fluid the larger the relative error of the actual count expected. This sort of dependence of the magnitude of error upon the expected magnitude of the determination is often discovered only by perusal of large sets of data offered only by careful record keeping and analysis. The benefits to be gained by the discovery of such a relationship are obvious. An adjustment in concentration may yield a test procedure very similar to the one used previously but with more accuracy.

C. Psychological benefits can certainly be derived from the Quality Control program. No one can discredit the pride of accomplishment which comes from successful completion of team effort. The entire laboratory must be on guard for sources of variation in the laboratory procedures. This sort of effort and continued surveillance lifts the pall of the routine. You might consider yourselves sharpshooters attempting to hit a definite target. Sharpshooters would certainly be bored if they could never look to see how well they were shooting.

III. Experiments Designed with Specific Goals in Mind

For emphasis we say again, the first step in any experiment is to find out about the total error. How big is it? This should be your first experiment, your first endeavor in your laboratory. This sort of experiment would play the role of the now famous or infamous, as you choose, Pennsylvania trials. This type of experiment which does not identify the sources of error may contribute a great deal to the field by simply pointing out that they exist.[3, 12]

A. An Experiment Comparing Two Methods of Measurement

The choice of the better of two methods of measurement of course will depend upon such factors as time and cost. These must be weighed under their own merits. We are concerned at present with the evaluation of the two methods in terms of which of the two is the more accurate. The most important single concept to be kept in mind with respect to this type of experiment is that of having a correct attitude. Usually this type of experiment involves a comparison of a new method with an old method. The typical attitude is to compare the two methods to see if the new method gives the same answer as the old, if not, throw all the blame on the new method. A more realistic attitude is to treat both methods as measurements subject to error either of which may be responsible for any observed difference in answers. This fundamental change in attitude will make a considerable difference in the method of analysis of the final results. In general, an experiment which involves the simultaneous measurement by two methods of a given unknown can often yield a great deal more information concerning accuracy and precision of both the methods than would be available by simple independent repetitions by the same method alone.

B. The Value of Experiments Designed to Obtain Information Concerning the Influence of the Level of the Unknown Quantity Upon the Variability of the Determination.[5, 6]

The usual goal in determinations which involve arbitrary concentration is to set the concentration so that the run of the mill or average or normal values are in the range of best accuracy. There is a possibility that a readjustment of thinking might be appropriate here. Perhaps the best accuracy should be sought in the area of important pathological deviations. In other words, the concentration should be such that in the event the unknown determination being attempted happens to be in the range of important pathological deviation it would be measured with the maximum possible accuracy. If it happens that the unknown is in the normal range the accuracy desired or necessary would not necessarily have to be so good. An awareness of the relationship between concentration and accuracy may make it possible to utilize two tests to compliment one another. One test which is accurate over a certain range of values and another test accurate over another range of values can be used simultaneously on the same specimen. One or the other of the two tests will therefore cover the unknown with required accuracy. This concept of complimenting tests, complimenting in this peculiar way, could not be considered unless one is error-conscious and aware of the effect of level upon variability.

C. Experiments Designed to Analyze Components of Error.[5, 6, 10, 11]

Before discussing a specific example a few comments are probably in order. Whatever method of analysis is used, whatever the sources of error which the method attempts to break out and consider, the main benefit to be derived from the experiment will be an awareness of the relative size of the various components of error. This relative size concept will give clues as to where one should focus his attention in attempting to improve the overall method. The general attitude in laboratories where statistical analysis is not usually done is to focus attention on the technical aspects of the determination and to attempt improvement where obvious technicological improvement seems reasonable and possible. Actually, this may not always be the most sensible and economical approach. It seems reasonable that one should focus attention on that component of error which is most important in the sense of its being the largest contributor to the total error. It does little good to reduce one source of error to its bare minimum even though it is technicologically simple to accomplish this when that source of error contributed a negligible amount to the total variation in the beginning. One might ask, "What component of variation, or error, should one attempt to separate out?" This depends upon *a priori* knowledge, the experience of the technologist, the chemist and the physicist. These are decisions that are in the realm of the technological field, not in the realm of statistical judgment. The discussion of specific types of designs of experiments depends equally as heavily upon the knowledge of statistics as upon the knowledge of the field. Therefore, no attempt was made to give a general method or even to give examples. There is really no general method except in the sense that one might consider a good textbook on design as incorporating a general method. We consider it dangerous even to give a few examples, because they may very well be copied as being recommended typical procedures. What follows, therefore, is a more or less schematic presentation of the

ideas that might possibly be incorporated into a designed experiment to analyze components of variation.

(1) Simple Factorial Experiments

One may wish to ascertain the contribution to total error due to the variability in the pipettes available. One may also wish to determine the effect of the use of two different reagents in a given determination. Both these experiments can be carried on simultaneously with the added benefit of gaining information concerning interaction between the types of pipettes and the types of reagents. Figure 1 gives a schematic presentation of the type of arrangement of observations that one might make to do this analysis.

Figure 1

BRAND OF PIPETTES

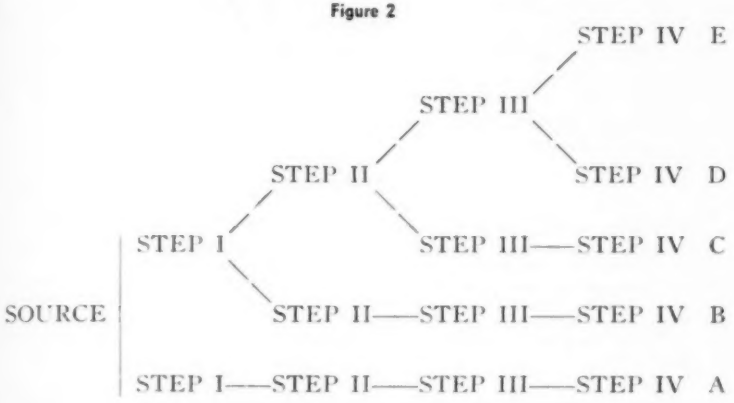
	A	B	C	D
REAGENT I	p_1 p_2 p_n			
REAGENT II	p_1 p_2 p_n			

In the column headed "Brand of Pipette A" one can visualize a random selection of pipettes from the source of pipettes of "Brand A." The numbers recorded within the cells under "Brand A" correspond to the use of reagent I and reagent II with each of the individual pipettes chosen. One would assume that all of the determinations throughout the entire table are determinations on the same material. A comparison of the measurements on the same pipette, but with different reagents will give an indication of the contribution to error variation due to different reagents considered over all brands of pipettes. A comparison of the average of the determination in each of the columns will give an indication of the variability contributed to the procedure by different brands of pipettes. A comparison of the observations with a single cell will give an indication of the variability of the determination which is due to the variability of the pipettes within a given brand when using a single reagent. It will be possible to analyze for interaction by seeing whether the average effect of the reagent over all of the brands is the same as it is for each of the individual brands. It may be possible that such an analysis will indicate that there is no such thing as a best overall reagent or a best overall brand but that a certain combination of brand of pipette and type of reagent will give the most accurate and least variable determination.

(2) Step Sequence Experiments

Many experiments are step-wise in the sense that first a certain step is performed and the results of that step are taken and worked on in step two and so on. It may be that one would wish to determine what part of the total error is contributed by each individual step. If one were to consider a simple 4-step procedure schematically, figure 2 indicates the very smallest and simplest possible design which would yield information about the contributions to error by each of the various steps.

Figure 2



The original unknown is first broken into aliquots and step I is performed on each and then on one of those steps II, III and IV are done routinely. On the other aliquot for which step I has been completed it is broken up into two equal parts and step II is performed on that and so on as indicated in the above figure. The lines corresponding to each possible sequence of determinations are listed from the bottom A, B, C, D, and E. Intuitively it is clear that some information concerning the total variability of the procedure could be obtained by comparing line A with an appropriate weighted average of lines B through E. Correspondingly, information concerning the variability in the procedure from step II onward can be obtained by comparing line B with an appropriate weighted average of lines C, D, and E. Finally, through manipulation, an estimate of the contributions to total error due to steps I, II, III and IV can be taken apart. Obviously, this is the smallest possible experiment which could give any information concerning these single components. The entire experiment starting with another sample from the same unknown should be replicated more often than is indicated here. There might be some reasons for estimating that particular component of error more accurately than one of the others. This sort of design could be used only under the simplest possible assumptions, that it, that there is no interaction between the level of the unknown at any step and the magnitude of error that might arise in the next step. Any additional complication of dependence of the steps as far as

the types of the error that might arise will necessitate a much more complicated design. No attempt has been made to discuss how one would set out to analyze the results of such a design. The goal has simply been to indicate that such experiments must be set down in a logical, orderly manner and a great deal of thought must go into their planning before starting out to study variations.

Certain aspects of a determination may appear to be part of a stepwise procedure when actually they are not. For instance, reading of temperatures, a reading of blanks, or obtaining the barometric pressure may be necessary steps in the experiment. Actually, they contribute to the final result only in the sense that they enter the formula for calculation. This sort of determination can be done over, and over and over again without respect to the other parts of the fundamental determination. Since this is possible one can determine the magnitude of the source of error as accurately as one wishes, more or less independently of the determination.

CONCLUSION

This has been a very sketchy indication of some of the principles involved in design. In view of the time available, no attempt has been made to give more precise, specific examples. The principles of good experimentation and design of analysis are readily available in texts. Many fields, such as agriculture, have used design and statistical analysis immeasurably to benefit their progress. Industrial chemistry is daily making use of these same concepts. The tools are available. They have more than proved themselves in fields very closely adjacent to your own. All that remains is a successful adaptation for their use in the clinical laboratory.

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THE FORCE OF VOLUNTARY COOPERATION*

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In showing its face to the world, America lately has permitted such blemishes as intransigence in the steel industry, quiz show scandals, and political temporizing at state and national levels to detract from more fundamental and appealing characteristics of our national life.

While deeply disturbed by these evidences of personal and group irresponsibility, I'll take a leaf today from Carlyle who said, "A man lives by believing something; not by debating and arguing about many things."

I'd like to state today my beliefs about the concept of voluntary cooperation, the lifeblood of the American Society of Association Executives, of the individual trade and professional associations in this country and of a great multitude of significant activities taking place throughout our nation.

I'm sure you all have your own definitions of voluntary cooperation. For the sake of establishing common ground, however, I'd like to offer this one of my own:

> "Voluntary cooperation is a social mechanism through which individuals and groups freely join forces to solve problems which are too big for individual action."

As you men and women here today appreciate more than most people, this mechanism of voluntary cooperation, along with the American form of competition, helps make our society unique. The spirit of voluntary cooperation has been manifest in our country for centuries. It is basic to our form of democracy. Yet it seems to me there is urgent need today to reawaken not only the American public to its importance, but to reflect its basic principles to nations abroad, particularly those who continue to weigh the relative merits of our democratic society as compared to its totalitarian challengers.

Seek Higher Expressions of Citizenship

To make a convincing demonstration to the world of the advantages of our system, we need first, to understand ourselves better—our heritages, traditions and democratic institutions—so that we can more effectively articulate the philosophical basis of our system; and second, we must find ways to develop new and higher expressions of citizenship in America. Mechanisms of voluntary cooperation, properly conceived and directed, will help serve both purposes.

Later on, I want to make specific proposals about the attainment of such goals, with particular reference to what the American Society of Association Executives can do to help. Initially, however, I'd like to sketch in some background and experiences which have helped shape my thinking.

Dr. Charles Malik, immediate past president of the United Nations General Assembly, and one of the wisest observers of American life, and of Communism, in an interview with *U. S. News and World Report*, gave this thoughtful analysis of the philosophical roots of our society:

* An address presented before the American Society of Association Executives, Boca Raton, Florida, November 1959. Published with permission from the A.S.A.E.

"You have in your own traditions certain invaluable beliefs about man and society and history and human destiny and the nature of God—beliefs that you should not be ashamed of, that you should feel free to export and to teach others. But first you must rediscover them yourselves and believe in them profoundly yourselves. Many of you have taken life too much for granted. Therefore, the great deposit of belief and conviction and interpretation of life which has come to you, you simply don't honor enough.

"My deepest fear—if you want it put bluntly to you—is that you don't know the infinite values that you have at the basis of your own civilization, and you don't believe in them enough to put them strongly to the rest of the world. You take life too easily. You take things too much for granted. You are not fighting for your values as much as your forebears were."

State Made Subservient to the Citizen

We are the beneficiaries of the fight for human liberty and justice which has been waged since the beginning of time. The American revolution of 1776 itself was a natural outgrowth of a struggle begun a century and a half earlier that sought to establish religious freedom and separation of church and state. The outcome of our subsequent political and economic American revolutions differed from those in Europe in that here people were put in charge of the state. Our divinely inspired Constitution made government subject to the consent of the governed. The key to the successful result was the division and dispersal of governmental power. The state was designed to remain the creature and servant of man.

Because of the challenges of the frontier, Americans from the beginning banded together in groups to accomplish tasks that were too big for the individual to handle alone. This urge for association extended to the business realm with the organization of the first Chamber of Commerce in New York City in 1768 and with the first trade association in Connecticut in 1853. These early trade organizations differed radically from those in the old world which were agencies of government or linked to government. Indeed, the New York Chamber was formed to resist governmental action that was considered damaging to the economic strength of the colony.

However, the concept of voluntary cooperation spread in many fields. One of the classical comments on the tendency of Americans to join together for common goals was made by Alexis de Tocqueville more than a century ago. He said:

De Tocqueville's Observation

"Americans of all ages, all conditions, and all dispositions constantly form associations. They not only have commercial and manufacturing companies, in which all take part, but associations of a thousand other kinds—religious, moral, serious, futile, extensive, or restrictive, enormous or diminutive . . . If it be proposed to advance some truth or to foster some feeling by the encouragement of a great example, they form a society . . . I have often admired the extreme skill with which the inhabi-

> tants of the United States succeed in proposing a common object to the exertions of a great many men, and in getting them voluntarily to pursue it . . . Thus the most democratic country on the face of the earth is that in which men have in our time carried to the highest perfection the art of pursuing in common the object of their common desires, and have applied this new science to the greatest number of purposes."

In our own lifetimes, we've watched the federal government become a gargantuan creature, which is increasingly costly and difficult to control. We can't expect to curb the further intrusion of government into business affairs if businessmen continue to lead the parade to Washington for handouts. Nor is government power going to be kept distributed and dispersed if states and local communities continue to look to the federal government for primary help on tasks which the communities should undertake themselves.

In my opinion, the steady drift toward big government can be arrested only if we implant the spirit of self-reliance and generate a renewed appreciation of voluntary cooperation as a mechanism for expressing this self reliance.

"The spirit of democracy," Milton S. Eisenhower once wrote, "shows itself not only in faith but in works." He urged that we make known the instances of " . . . Free citizens in a free land voluntarily coming together to solve their own local problems."

These examples, he said, should be put before Americans so that their own faith and their practice of democracy might be strengthened and extended.

President Wilson expressed the principle succinctly when he said, "The most powerful force in the world is the spontaneous cooperation of a free people."

I would now like to offer several examples of the effectiveness of voluntary cooperation in meeting some problems of our time. I've had the good fortune—as have some of your members here today—to have been actively associated with these particular endeavors. So I speak from a background of firsthand knowledge and understanding of the magnitude of the problems that had to be faced.

In introducing this first example, I'd like to quote Henry Luce, the publisher, who recently observed:

> "Organization with freedom, however untidy, is in every way superior to organization by tyranny, however benevolent."

A powerful example of the truth of this observation occurred during World War II. As a young man only recently transplanted in Detroit after cutting my eye-teeth in trade association affairs in Washington, I undertook to help the automotive industry organize a cooperative mechanism, the Automotive Council for War Production. The Council's purpose was to expedite the output of guns, tanks and planes to help our nation carry on a war on two fronts.

Let me give you a bit of background on the Council. It illustrates the extent to which highly individualistic men and companies in one of America's major industries were capable of submerging personal and competitive interests to the public interest.

At a meeting of the Automobile Manufacturers Association in New York in October, 1940, William S. Knudsen was to be given an award. He had recently resigned as president of General Motors and was heading up the nation's defense production. He told the group he had something of great import to discuss. He prefaced his comments by revealing that General Hap Arnold, chief of the Air Corps, had just returned from England where he had observed the blitz. General Arnold was convinced the nation which produced the most medium and heavy bombers would be the ultimate victor. Knudsen himself had just completed visiting all our aircraft plants. He said the aircraft companies had expanded 10 to 20 times and were in no position to take on an additional load.

Mr. Knudsen said to the auto industry, "You've got to help." Before the day was over he had a pledge from the whole industry that it would subordinate its regular activities to the mass production of airplane parts and components for the defense program. Thus in 1940 began the Automotive Committee for Air Defense. The cooperative mass productive might of the motor car industry was put behind the bomber program. Jimmy Doolittle, then a major, was given the job representing the Air Force in the task of getting the aircraft and automobile industries working together.

Teamwork for Victory

The motor vehicle and parts companies learned that they could plan together, work together, build together, and exchange production know-how with each other for the common cause. In addition to making airframe sections, the automobile industry was called upon to produce three out of every four of the necessary aircraft engines.

Following Pearl Harbor the industry organized the Automotive Council for War Production. The industry proceeded with its organization despite a threatening telegram from the Department of Justice that it might violate anti-trust laws. Through ACWP, companies cooperated as no industry had ever before cooperated to preserve human freedom. They were fulfilling their pledge that our armies and those of our allies "would not lack for one gun, one plane, or one tank which our skill and ingenuity can produce."

The Nazis came to a belated recognition of the worth of such a cooperative pattern. Not until February 1943—more than 41 months after Germany began total war—did Albert Speer, Nazi minister of munitions, order German industrialists to pool their experience and share their production methods. This was a tacit admission that enforced cooperation had not worked in Germany's completely collectivized state.

As a historical footnote on this Nazi failure, I would like to make a comment on the German cartel system, which was a basic part of German Big Business in peacetime. The first German cartel was organized in 1862, less than a decade after the founding of the first trade association in America. Before Hitler came to power in Germany, the number of cartels had grown to about 2,500.

Comparison of Cartel and Trade Association

The cartel and trade association are alike in that neither actually runs any business undertaking. Each has members who retain their

individual autonomy and who direct and control the association or cartel as they would a business in which they had common ownership.

But the American association cannot require its members to perform or refrain from performing certain business acts. Both membership and cooperation are voluntary.

On the other hand, the cartel is built on a contract or agreement, which is recognized and enforced by law. Membership is voluntary but cooperation is not. The members are bound by certain articles of agreement and are penalized if they violate those articles. A contract between members determines selling prices, allocation of production and markets.

The real test of the cartel is its ability to influence the spread between cost and price of a commodity.

After the defeat of Germany in World War I, and the advent of Social Democracy in Germany in November of 1918, the attention of the government was naturally centered upon the organization of industry.

In each case, the socialization plan was built around the cartel as the key unit. By the time Hitler took over, German industrialists had been thoroughly cartellized and an intimate relationship already existed between government and industry.

Hitler completed the process. All business was required to join the cartels and all of these were brought under the direct and absolute jurisdiction and control of the government Department of Economics.

In the acid test of World War II, compulsory cooperation failed to match voluntary cooperations.

With a vast flood of armaments rolling from America's automotive and other plants, a steel ring was steadily forged around Hitler's crumbling empire.

In 1945, a Detroit newspaper editor, Malcolm Bingay, stood beside General Devers looking over Munich's smoking ruins. The general remarked to Mr. Bingay:

Doing the Impossible

> "Never in the history of the world has any one group of men contributed so much to the victory as that group of Detroit industrialists. They forgot all about their rivalries in business under that Automotive Council. They reminded me of a fighting football team. Never was there such unity of purpose. They not only gave us what we wanted, they frequently suggested things that we did not dream of ever having. Because they had the know-how, the initiative and energy, we gave them the impossible things to do. They did them, and set a pattern."

Some would discredit the voluntary cooperation approach on the basis that modern problems are of such magnitude that government alone can get the pressing jobs handled.

Our age is confronted with problems of great complexity, growing out of the mobility of people and the urban sprawl. But the solutions to our pressing problems call for a higher order of citizenship and proper mechanism for expressing citizenship responsibilities. Dependence on government agencies alone will not be sufficient.

Too Big for Government Alone

The traffic accident and fatality problem is an example of a major

public and social condition that did not yield to the efforts of government alone. With the use of motor vehicles growing at a very rapid rate, the number of persons injured or killed in or by motor vehicles pyramided between the world wars. Not until the automotive industry joined with hundreds of other groups at the national, state and local levels did the traffic fatality tide begin to recede. A consistent, concerted effort calling for more effective education, enforcement and engineering helped produce these rsults:

In 1937, the year before the automotive safety program was launched, traffic fatalities averaged 14.7 per 100 million miles of motor vehicle travel. Last year, largely as the result of intensive cooperation of groups and individuals at all levels, traffic fatalities declined to 5.6 persons per 100 million miles of travel. For the first seven months of 1959, the traffic fatality rate is down to 5.1.

Here's another way to look at the reduction in human toll: If cars, trucks and buses were killing people at the 1937 rate, instead of 37,000 persons being killed—regrettable as that total is—the current total would be close to 100,000 persons.

Detroit Reduces Traffic Toll

In the city of Detroit, for example, the job of educating the public in safer driving was largely left to the police department. Then Detroit business and industry began to concern themselves about the problem. Industry leaders organized the Detroit Traffic Safety Association. The Association works hand-in-hand with the police, the courts, the schools, newspapers, radio, television and other groups in promoting safe driving.

Some 1,881 fewer persons were killed in Detroit traffic during the 17 years since the Association was formed than in the previous 17, despite a 78.8% increase in motor vehicle registrations.

As the awesome magnitude of modern problems confront us, such as how to meet our educational needs, some people point admiringly to all-out government support of such activities in the Soviet Union. They suggest we need to adopt similar practices here.

We Over-emphasize Russian Ideology

I believe one of the great threats to our way of life is that we'll overrate the effectiveness of the Russian ideology and permit it to infiltrate our own. To guard against this tendency, we must reaffirm our belief in freedom, democracy, individualism and voluntary cooperation—the cornerstones of our great system.

We have some decided advantages over the Russians. One is that we know how to get things done through voluntary cooperation. This is a real advantage in a rapidly changing society.

As a social tool, voluntary cooperation possesses remarkable qualities. It is sufficiently flexible to embrace a variety of different problems, large and small, simple and complex; it is adaptable to shifting needs and conditions; it can be utilized for short-range purposes or can be continued over an indefinite period of time. The more actively one participates in voluntary cooperative activities, the greater his personal satisfaction. Even when the particular mechanism is brand-new, it can produce results more promptly and effectively than older social forms.

provided sufficient energy, vision, faith and determination are applied.

To illustrate the point let me briefly relate one recent Detroit example in which the tool of voluntary cooperation played a primary part in changing the attitudes of the voters toward the urgent and somewhat neglected problem of public education.

Meeting an Educational Crisis

As with many large urban communities, Detroit was confronted with mounting enrollments, obsolete school buildings, an inadequate supply of teachers and insufficient funds to meet current operating expenses. Because of a 15 millage tax limitation, it has been necessary from time to time to secure from Detroit voters special consent for extra school funds. In 1957, voters turned down by a 3 to 1 vote the Detroit Board of Education's request for monies required for full school operation and plant expansion.

This turn-down was in face of such stark facts as these: nearly 24,000 high school students could be accommodated only in half-day sessions, various school buildings were obsolete and frighteningly unsafe, and an increased enrollment of 30,000 more pupils was expected in the next decade.

To meet the impending educational crisis in America's fifth largest city, the Board of Education authorized the appointment of a Citizens Advisory Committee on School Needs. This was nearly a year before the launching of Sputnik shocked the nation as a whole into paying more attention to the educational needs of our youth.

I had the rich and satisfying experience of heading this committee during the eighteen months of its operations. It deepened my faith in the processes of democracy.

The Detroit educational job was an imposing one. Both parents and the community as a whole had lost contact with the schools. Lack of facts and contradictory information had plagued the previous effort to secure needed community action.

We had to roll up our sleeves and get on with the job. For the citizens committee 273 persons were selected, representing the different geographical sctions of the city. They were representative, too, of different nationalities, economic groups, religious faiths and political parties. In addition to a city-wide committee, we had eight separate regional committees, so the problems peculiar to particular sections of the city could be isolated and treated individually.

I made only three requests of my fellow committee members. One was that each attend meetings for himself as far as possible. The second was that each person speak his mind as an individual citizen, not as a representative of the organization that employed him, or with which he was actively affiliated. The third, that we determine and study the facts before we made any recommendations.

A surprising number of our meetings registered almost perfect attendance. Task forces of citizens inspected 287 school buildings, interviewed teachers, reviewed curriculum schedules, worked with outside consultants and analyzed Detroit school financing in light of sound business practices.

The facts we collected and the recommendations we made covered

more than 1,000 pages, filling six volumes. The most careful planning went into making the findings available to the community at large through newspapers, radio, television, luncheon club speeches, and other media.

As the Spring election of 1959 approached, ill tidings came from suburban communities in the greater Detroit metropolitan area. Voters were in a cautious mood; proposal after proposal for adding to the cost of local government, including school costs, were voted down. In a city that was economically depressed, the Detroit Board of Education was making a double barrelled request of the voters; the citizens were asked to approve both a 3 mills tax increase plus a $60,000,000 bond issue.

By the April 6 election, however, the case for better Detroit schools had been registered with the electorate. The voters clearly understood the issues. The facts were never seriously questioned because of the unimpeachability of the citizens committee and the obvious thoroughness of its research. When the returns were in, the work of the voluntary citizens' organization was roundly endorsed by the voters, who favored the proposals by margins of eight to five on the millage increase and by eleven to eight on the bond issue.

The mechanism of voluntary cooperation is indeed flexible and adaptable to a variety of situations. Basically the same approach some of us applied to the school problems of a single community we currently are applying to a larger area, the state of Michigan, and to correct a larger problem—political apathy.

Michigan is Politically Bankrupt

The impression created throughout the nation this year is that the state of Michigan is bankrupt. It is, but the state's financial troubles are not the basic problem; they can be readily solved. The real problem is that the state is politically bankrupt.

Because of time limitations, I will not try to deal today with all of the complexities of Michigan's current crisis. The origins of the problem go back many years when the state's two major parties became, in effect, captive of Big Business interests and Big Union interests. Many citizens eventually lost interest in either party. This year the financial crisis starkly revealed how narrow, selfish and excessively partisan were the programs and goals of the state Republican and Democratic party leaderships.

In an effort to re-interest the citizens in the state government issues, a few of us several months ago organized a non-partisan Citizens For Michigan group to study and make recommendations on the fundamental problems of the state.

We've invited citizens to work together not as Republicans or Democrats, nor as representatives of particular social or economic groups, but as citizens. We've limited to $100 the contribution that any individual or organization (except for foundations) can make.

Same Approach as on School Problem

We're using basically the same approach that was used by the Detroit Citizens Advisory Committee on School Needs. A Citizen For Michigan central committee will prepare a statement of specific problems, and

ask local groups to review the statement and comment on it. In this way, we expect to reflect both state-wide and local viewpoints, much as we did in Detroit by using the city-wide and regional approach.

Next both the state and local groups will study facts in each specific problem area. On the basis of thoroughly informing themselves as to the facts, the state and local groups will arrive at preliminary recommendations. After further review at the state and community level, we expect to make final recommendations late next spring, backed by what we hope will be a reasonable unanimity of viewpoints of the active participants on the long-range solutions.

I believe that Citizens For Michigan can perform a vital function in a state plagued by a break-down of the democratic processes of government. It is clear that the present party leadership has temporized because the will of the citizens was not being effectively expressed. By making it possible for citizens first to inform and then to express themselves on the issues, Citizens For Michigan can help the state of Michigan emerge from the bogs of political expediency and resume its progress on the basis of soundly conceived programs.

Frustration of Citizens Generally

Citizens For Michigan can have a larger meaning, too, if it succeeds in establishing a successful pattern for citizen participation through voluntary cooperation in government affairs which can be applied elsewhere. The opinion polls conducted by Samuel Lubell and others have uncovered a deep-rooted feeling of uneasiness, frustrations and helplessness on the part of the American people. They are conscious of the drift and indecision at state and national levels, yet they are floundering as to what they individually can do about it. They are seeking leaders of faith, courage and understanding who can give direction to their yearnings for greater spiritual satisfaction, and they want to work toward higher personal and national goals.

The challenge to America is well expressed in the words of Alfred North Whitehead, the British scientist and philosopher. He wrote:

> "We must produce a great age, or see the collapse of the upward striving of our race."

Our age and the magnitude of our problems requires that we develop men of character and ability who will exercise responsible leadership in their communities—the logical training ground for eventually assuming even greater responsibilities at the national and international level.

We all are acutely conscious that progress in the social sciences has lagged behind technological progress. We need to develop voluntary cooperative mechanisms that well enable metropolitan communities to eradicate slums and arrest the encroachment of blight, and provide better programs and opportunities for their youth.

Those of us who have fled to the suburbs should assist in the improvement of the environment of those living in the midst of the noise, smell and ugliness of the city. If we deplore the spread of juvenile delinquency, we might ask ourselves, "What have I personally contributed to providing wholesome recreational or vocational outlets for our youth? What can I do to help combat disease and social tensions among their elders?"

The answers are not easy. We do not discharge our obligations by making annual financial contributions to the Community Chest. We cannot satisfy our consciences by shunting the job off on social agencies or by demanding that some government agency do something. We give lip service, or money, or opinions when we ought to give ourselves.

We in American Motors who are primarily concerned with the company meeting its obligations as a corporate citizen were heartened two years ago to find the United States Junior Chamber of Commerce entertaining similar views about the need for developing active leadership in the community field.

After ascertaining in detail the Jaycee's objectives, American Motors has decided to make their comprehensive community development program a major beneficiary of our corporate contribution budget.

The Jaycees, through their 4,200 local chapters, are going about the community development assignment much as other voluntary cooperative groups have done in the instances previously cited.

In each community where such an activity is underway, the local Jaycee chapter reviews the major community problem, studies the facts pertaining to the problem, then organizes public support to carry out the recommended improvement program.

To stimulate the local chapter effort, American Motors annually puts up three cash awards for the chapters that do the most effective job in getting the community as a whole behind a priority project. We also make it possible for representatives of winning chapters in each of the 50 states and the District of Columbia to attend a national seminar at which the chapter representatives exchange experiences as to the techniques that produce positive community action.

The Junior Chamber of Commerce International, an organization that parallels the United States Junior Chamber, with active organizations in 55 countries, has become interested in undertaking a community program modeled on the Jaycee activity here. My company has recently agreed to provide the "seed corn" to enable the JCI to add to its Miami Beach headquarters stall and adapt the Jaycee-American Motors program for use abroad.

Spread of the voluntary concept abroad may be particularly timely because of the concern of member nations of the United Nations about economic problems at the community level.

The United Nations has recently authorized studies pertaining to community development, which it defines as "a process designed to stimulate and promote conditions for social, cultural and economic progress by coordinated efforts which involve primary emphasis on responsibility and action at the local level."*

As far as the U. S. foreign aid policy is concerned, opportunity exists for America to demonstrate the efficacy of self-assistance, backed by sound technical help, rather than primary reliance on financial aid bestowed on the central government of underdeveloped nations.

Since getting more deeply into the voluntary approach in the community field, we've been gratified to learn about somewhat similar programs of other American organizations in this area. One of them is the

* United Nations Series on Community Development, "Aspects of National Community Development in Asia," May, 1959.

community achievement contest which the General Federation of Women's Clubs carries on in cooperation with the Sears-Roebuck Foundation. Another activity is sponsored by the Union Electric Company which makes "Planned Progress Awards" to Iowa, Illinois and Missouri, towns of less than 7,500 population served by the utility company. Still another company, the Lane Bryant stores, annually recognizes the achievements of individuals anywhere in the nation who have contributed outstanding personal effort to volunteer community service.

Because the health of a democratic society may be measured by the quality of functions performed by private citizens and their voluntary organizations, I believe that still greater recognition is due this significant aspect of American life.

There is no organization in existence at the present time whose primary purpose is to foster the greater understanding of the role of voluntary cooperation. Therefore, I'd like to propose the establishment of the American Foundation for Voluntary Cooperation.

The American Foundation for Voluntary Cooperation would provide encouragement and recognition to individuals and groups that are making significant public service contributions through the instrument of voluntary cooperation.

Here's the way I view the structure of the organization:

1. The Foundation would consist of a Board of Trustees composed of nationally outstanding and representative men and women who are recognized for unselfish efforts in furthering cooperative endeavors in economic, educational, civic, religious, vocational or community life.

2. Advisory committees would be created to make recommendations to the Trustees and staff on identifying meritorious cooperative programs, choosing recipients of awards, publicizing outstanding examples of voluntary action, etc.

3. A small permanent staff would be established to assist in carrying out the Foundation's objectives.

Here are some mechanics that might be employed to promote the Foundation's goals:

A. Annual Recognition Dinner.

An annual dinner would be held at which monetary awards, or other forms of recognition, would be given to individuals, groups or social mechanisms which (1) have made important contributions to the fields of voluntary cooperation, or (2) have used the tools of voluntary cooperation to attain outstanding social, economic or cultural results. The awarding of Civilian Merit Medals to private citizens selected for their outstanding work in leading or establishing cooperative activities could be an annual feature of the dinner.

B. Awards

Awards of scholarships, teaching fellowships, grants, plaques and citations would be made at the local, regional and national level.

C. Public Information

The source material gathered by the Foundation staff would be freely available for television and radio programs, motion pictures, books, magazine articles, newspaper feature stories, etc.

As association executives, you men and women rank high among

the practitioners of the art of meeting common goals by stimulating concerted, voluntary effort. I hope your interest in achieving greater status for the voluntary approach extends beyond your particular industry or profession, and, indeed, beyond the association field generally.

If my assumptions are correct, I would like to suggest that you appoint a special committee to determine the feasibility of the American Society of Association Executives joining with others in the establishment of an American Foundation for Voluntary Cooperation.

If you feel such a Foundation has merit, would you be willing to help the future organizers identify those companies and individuals in your own industries, or in your home communities, who might be willing to take an active part in such an endeavor?

Why am I as a citizen interested in furthering such a program? Because I firmly believe that our best means of countering the Communist ideology lies in demonstrating the effectiveness of freedom—religious freedom, political freedom, economic freedom. We need to demonstrate by example at home and abroad that persuasion and voluntary cooperation lead to greater social and economic gains than compulsion and collectivism.

America's greatest shortage is trained leaders. Leadership is particularly short in areas where social progress has lagged behind technological advancement. We need, therefore, a new and dramatic device that will provide encouragement and recognition to individuals and organizations that have made outstanding contributions to these fields.

In conclusion, let me point out the tremendous stakes that are before us. Internally our nation faces a persistent demand for multi-million dollar government programs to conquer our new economic and social frontiers. If we are to arrest the trend toward an ever-bigger and highly centralized government bureaucracy, we individually must join with others in effective voluntary programs.

Externally our efforts to build a peaceful world are being distorted by a clever and voluble international propaganda machine run from the Soviet Union. Will our own voice be clear and strong enough to be heard over the alluring and distracting noises from the Kremlin?

We are at the point in our world destiny where the methods and ideals that conquered the mountains and deserts are needed to win the minds and hearts of men. We must see that the truth about what we stand for and are doing is effectively presented to the people around the globe.

We must give the nation and the world a convincing demonstration of the powers of free men, cooperating with each other, by building a society in which we will achieve a higher expression of our spiritual, political, economic and social principles. Through example and more effective communication we must build a still greater age in America and in the world or see our past accomplishments and future opportunities perish before the ignorance and envy of others who yearn for what we have.

I believe voluntary cooperation is an indispensable means of building a greater age for all mankind, because it is the practical expression of brotherhood in action.